This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: ______________________

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

BEST AVAILABLE COPY

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :	A1	(11) International Publication Number: WO 97/35978
C12N 15/12, C12Q 1/68, C07K 14/47, 16/18, G01N 33/574, 33/577		(43) International Publication Date: 2 October 1997 (02.10.97)

(21) International Application Number: PCT/US97/05059

(22) International Filing Date: 27 March 1997 (27.03.97)

(30) Priority Data:
08/623,428 28 March 1996 (28.03.96) US

(71) Applicant: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Office of Technology Transfer, National Institutes of Health, Suite 325, 6001 Executive Boulevard, Rockville, MD 20852 (US).

(72) Inventors: LINEHAN, Marston, W.; 7009 Tilden Lane, Rockville, MD 20892 (US). LERMAN, Michael, I.; 1618 Martha Terrace, Rockville, MD 20892 (US). LATIF, Farida; 803 Gabriel Court #311, Frederick, MD 21702 (US). ZBAR, Berton; 4400 Oxford Street, Garrett Park, MD 20896 (US).

(74) Agents: FEILER, William, S. et al.; Morgan & Finnegan, L.L.P., 345 Park Avenue, New York, NY 10154 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: PARTIAL INTRON SEQUENCE OF VON HIPPEL-LINDAU (VHL) DISEASE GENE AND ITS USE IN DIAGNOSIS OF DISEASE

(57) Abstract

The Von Hippel-Lindau (VHL) disease gene and its corresponding cDNA are disclosed. Methods for detecting carriers of the VHL disease gene using probes derived from the VHL disease gene sequence are described. Pharmaceutical compositions and methods of treating diseases related to the VHL gene are also disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

TITLE OF THE INVENTION

PARTIAL INTRON SEQUENCE OF VON HIPPEL-LINDAU (VHL) DISEASE GENE AND ITS USE IN DIAGNOSIS OF DISEASE

FIELD OF THE INVENTION

The invention is in the field of tumor suppressor genes. More specifically, the invention relates to the Von Hippel-Lindau (VHL) disease gene and its corresponding cDNA and to methods for detecting carriers of the VHL disease gene using probes derived from the DNA sequences of the present invention.

BACKGROUND OF THE INVENTION

Von Hippel-Lindau (VHL) disease is a familial cancer syndrome. This disease is an autosomal dominant disorder and patients who are heterozygous for mutations in the VHL disease gene are predisposed to a variety of cancers, the most frequent being hemangioblastomas of the central nervous system and retina, renal cell carcinoma (RCC) and pheochromocytoma. The multisystem character of the illness, combined with the fact multiple tumors may form in each target organ, produces considerable morbidity and mortality as evidenced by the reduction in life expectancy of affected individuals to 49 years (McKusick, V.A., Mendelian Inheritance in Man (1983) Johns Hopkins University Press, Baltimore and London, p 534-535). Although the prevalence of VHL disease is only 1 in 36,000, because of its late onset most individuals have children before they realize they have inherited VHL disease. For many years, the only method of presymptomatic or prenatal diagnosis of the disease has been periodic examination of the eye, brain, and abdomen in all asymptomatic members of VHL families. Unfortunately, examination of all target organs is required to ensure detection of disease that may be limited to a single organ. In addition to the obvious inconvenience and the cost of these examinations, they have the additional

drawback that they may not yield definitive diagnostic information. Therefore, in order to develop a method which allows the unequivocal diagnosis of VHL disease in individuals at risk, researchers have focused intensive efforts on identifying and isolating the VHL disease gene.

Results of this research have shown that the VHL disease gene is a member of the family of tumor suppressor genes (Tory, K. et al. J. Natl. Canc. Inst. (1989) 81:1097-1101; Maher, E.R. et al. J. Med. Genet. (1990) 27:311-314) and that it behaves in accordance with Knudson's theory of human carcinogenesis (Knudson, A., Proc. Natl. Acad Sci. USA (1971) 68:816-823). In addition, the identification of DNA markers tightly linked to the VHL disease gene has allowed localization of the VHL disease gene to human chromosome 3p25-p26. (Hosoe, S. et al. Genomics (1990) 8:634-640; Maher, E.R. et al. Genomics (1990) 8:957-960; Glenn, G.M. et al. Hum. Genet. (1990) 87: 207-210, Latif, F. et al. Am J. Hum. Genet. (1992) 51 (suppl.) A63; Tory, K. et al. Genomics (1992) 13:275-286; Richards, F.M. et al. J. Med. Genet. (1993) 30:104-107); Seizinger, B.R. et al. Nature (1988) 332:268-269; Seizinger, B.R. et al. Proc. Natl. Acad. Sci. USA (1991) 88:2864-2868 and Vance J.M. et al. Am J. Hum. Genet. (1993) 51:203-209)). Recently, Glenn et al. (Glenn, G.M. et al. JAMA (1992) 1226-1231) have used DNA markers flanking the VHL disease gene as probes to detect linkage to the VHL disease gene via restriction fragment polymorphism analysis of DNA isolated from individuals who are members of families at risk for VHL disease. Although this DNA polymorphism method results in enhanced accuracy of identification of carriers of VHL disease gene, the method is inherently flawed in that DNA polymorphism analysis does not detect the VHL disease gene itself. More recently, a gene located in the VHL region has been cloned (Latif, F. et al. Cancer Res. (1993) 53:861-867). However, this gene was found to detect no mutation in VHL patients and thus, there are currently no available methods which can identify

carriers of the VHL disease gene with 100% accuracy. However, the recent identification and isolation of the VHL disease gene (Latif et al., Science, (1993) 260:1317-1320) and its corresponding cDNA should allow the development of diagnostic methods which provide unequivocal detection of carriers of the VHL disease gene.

SUMMARY OF THE INVENTION

The present invention relates to the von Hippel-Lindau (VHL) disease gene and its corresponding cDNA.

The invention further relates to methods for detecting carriers of the VHL gene. The first method comprises analyzing DNA of a subject for mutations of the VHL disease gene associated with VHL disease or other diseases, including, but not limited to, sporadic renal cancer, lung cancer, uterine cancer, breast cancer, testicular cancer, ovarian cancer, adrenal tumors, brain tumors, lung tumors or other cancers.

The second method comprises analyzing RNA of a subject for mutations or alterations in the VHL-specific mRNA associated with VHL disease or other diseases, including, but not limited to, sporadic renal cancer, lung cancer, uterine cancer, breast cancer, testicular cancer and ovarian cancer.

The third method comprises analyzing protein of a subject for alterations in VHL protein expression associated with VHL disease or other diseases, including, but not limited to, sporadic renal cancer, lung cancer, uterine cancer, breast cancer, testicular cancer and ovarian cancer.

The invention also encompasses recombinant VHL proteins derived from the VHL cDNA and antibodies directed against said VHL proteins or peptides derived therefrom.

The invention further relates to a method for treating a carrier of the VHL gene in which an expression vector containing a nucleic acid sequence representing the wild-type VHL gene is administered to the carrier.

The invention also provides a diagnostic kit for detecting carriers of the VHL gene. The kit comprises purified and isolated nucleic acid sequences useful as PCR primers in analyzing DNA or RNA for mutations of the VHL gene associated with VHL disease and diseases related thereto, including, but not limited to, sporadic renal cancer, lung cancer, uterine cancer, breast cancer, testicular cancer and ovarian cancer.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Figure 1 shows a genetic and physical map of the chromosome 3p region encompassing the VHL gene. Genetic and physical distances between selected markers are shown in centiMorgans and kilobases, respectively. The location of selected cross-overs is indicated by crosses. Panel B shows the 160 kb cosmid and phage contig covering the VHL region. An enlarged restriction map of cos3, cos11, and phage p191 detailing the position of g7 cDNA isolated by screening a λgt11 teratocarcinoma cDNA library with a conserved 7kb fragment from the centromeric end of cos11. The beginning of the smallest constitutional deletion is indicated by an asterisk and line. Restriction sites: B, Bam Hl; E, Eco Rl; N, Not I; Nr, Nru I; M, Mlu I.

Figures 2A and 2B: Figures 2A and 2B set forth a Northern blot analysis of the expression of the VHL gene represented by g7 cDNA in various human tissues. Figure 2A shows a low resolution blot containing 2 μg poly A^+ mRNA. The tissues are indicated above the lanes. Figure 2B shows a high resolution blot containing 1 μg of poly A^+ mRNA from: lane 1, fetal brain; lane 2, adult brain; lane 3, fetal kidney; lane 4, adult kidney; lane 5, cerebellum; lane 6, adult adrenal; and lane 7, prostate. The sizes of the transcripts were determined by the position of the 28S and 18S rRNA bands.

Figures 3A, 3B and 3C: Figures 3A, 3B and 3C show detection by Southern blotting analysis of rearrangement mutations in constitutional DNA of VHL affected patients using g7 cDNA as probe. Figure 3A shows DNA from lymphoblastoid cell lines of 7 unrelated VHL patients was digested with EcoRI and analyzed by standard blotting procedures. The normal invariant band is about 20 to 22 kb, the sizes of the aberrant bands probably resulting from intragenic deletions range from 4 to 25 kb. The patients code numbers are indicated above the lanes. Figure 3B shows DNAs from lymphoblastoid cell lines of pedigree members from a new mutation family (coded "S") digested with DraI, HindIII, and PstI. The pedigree with the position of the affected (filled circles) and predicted (hatched circle) members is shown. Males are represented by squares and females by circles. Figure 3C shows genetic transmission of the mutant allele (the aberrant band) in a regular VHL family (coded "P"). The DNAs were digested with EcoRI and analyzed by Southern blotting; the pedigree is shown.

Figure 4: Figure 4 shows a Southern blot analysis of genomic DNA of VHL patients (only the initials of each patients name are given). The DNAs were digested with EcoRI and probed using different regions of g7 cDNA. Panel A: Total g7 cDNA probe; Panel B: 5' end probe, nucleotides 3-146; Panel C: 3' end probe nucleotides 1277-1600.

Figure 5: Figure 5 shows the results of polymerase chain reaction-single stranded conformation analysis (PCR-SSCP) of the genomic DNA of VHL patients with the 8 bp insertion mutation (Table 1). Portions of the DNA sequencing gels are shown that display normal and 714insTTGTCCGT mutation sequences. The DNA sequence is of the antisense strand; therefore, the inserted bases are 5'-ACGGACAA-3'. Adjacent to the sequencing ladder are shown the positions of the insertion, and the nature of the

insertion, as predicted from the sequence.

Figure 6: Figure 6 shows the results of a "zoo" blot illustrating evolutionary conservation of the putative VHL gene. The g7 cDNA shows cross species homology to DNA from mammals, birds, fly, and sea urchin. Lanes: 1, human (Homo sapiens); 2, chimpanzee (Pan troglodytes); 3, macaque (Macaca fascicularis); 4, cow (Bovis domesticus); 5, rat (Rattus norvigicus); 6, mouse (Mus musculus); 7, chicken (Gallus domesticus); 8, frog (Xenopus laevis); 9, fly (Drosophila melanogaster); 10, sea urchin (Strongylocentrotus purpuratus); and 11, yeast (Saccharomyces ceriviseae).

Figures 7A, 7B and 7C: Figures 7A-7C show the RNase H mapping of the VHL mRNA. Figure 7A sets forth a Northern analysis of the RNase H digest of the VHL mRNA: 1-undigested RNA: 2-RNase H digest with oligomer 1: 3-RNase H digest with oligomer 2. Probe-extended exon 1 (bases 1-553; Latif, et al., 1993b). Figure 7B sets forth the same plot probed with exon 3 VHL group 7 cDNA (bases 740-1810). RNA markers: 0.24-9.5 kb RNA ladder (Gibco-BRL) human 28S (5000 nt) and 18S (2000 nt) rRNAs: Figure 7C shows the alignment of the VHL group - cDNA and VHL mRNA according to RHase H mapping; Oligomers 1 and 2 are represented by black boxes, exon 1 sequences are shown as hatched bars, exon 2 - black bars, exon 3 - open bars. Putative reading frame and scale (in kb) are shown below.

Figures 8A, 8B and 8C: Figures 8A-8C show the identification of the transcription initiation sites. Figure 8A sets forth the templates and probes used for RNase protection assays. Genomic DNA is represented by solid line, pBluescript II SK vector is represented by an open bar, RNA probes are represented by dashed lines (with the end nucleotides numbered from VHL mRNA transcription start

site +1). Probe numbers are shown in the right column. T3 and T7 promoters and their orientation are indicated. Filled bars represent protected fragments. Figure 8B sets forth an RNase protection assay using probes 1, 2, 3 and poly(A)' RNA from the 293 cell line. 1, 2 - probe 1 hybridized to 293 RNA (2μg): 3 - probe 1 and yeast tRNA (10 μg): 4-probe 2 and yeast tRNA; 5.6 - probe 2 and 293 RNA. 7-probe 3 and yeast tRNA; 8.9 - probe 3 and 293 RNA. 'Century markers' (Ambion): 500: 400: 300: 200: 100 nt C- RNase protection using probe 5 and 293 poly(A)'RNA 1 - hybridization of the probe 5 and yeast tRNA: probe 5 and 293 RNA. Markers: protected fragments obtained after hybridization of the control sense RNA (probe 4) and probes 5: 6: 7 or 8 (194:182, 170 and 147nt, respectively).

Figure 9: Figure 9 represents the identification of the VHL promoter region. Luciferase activity (right column) was compared to those for full length construct (residues -468/-195) which represents 100% activity in 293 cells (mean value). Restriction map of the 5' flanking genomic region is shown at the top of the Figure. The positions of transcription initiation and first methionine AUG condon are indicated.

Figures 10A and 10B: Figures 10A and 10B depict VHL minigene expression in UMRC 6 cells. Figure 10A describes expression constructs used for stable transfection of the UMRC 6 cell line. VHL sequences were shown as black bars, vector sequences - as open bars and solid lines. Predicted transcripts from VHL transgene represented by dashed line (size is indicated). Figure 10B describes Northern analysis of the expression of the VHL transgenes. Total RNA was isolated from four pools each containing 40 to 50 colonies transfected with different expression constructs: (1) pRc-HAVHL; (2) original UMRC 6 cells; (3) pRcp VHL3U; (4) pRcpVHL; (5) pRcpVHLm. Arrows indicate endogenous

expression, double arrows - exogenous. Note: Previously, the size of the VHL mRNA on Northern blots was calculated as 6 to 6.5kb (Latif, et al., 1993b). In this study, the size of the VHL mRNA was defined more precisely as 4.4 to 5.0kb (depending on conditions of electrophoresis). 0.24 to 9.5kb RNA ladder (BRL) and 28S/18S human ribosomal RNA was used as a reference.

Figure 11: Figure 11 shows an analysis of the UMRC 6 clone 4 transfected with pRcpVHLm. Panel A sets forth a Southern blot: 1.2 - HindIII digest: 3, 4 - HindIII/EcoRI digest: 1, 3 - original UMRC 6 cell line: 2, 4 - UMRC 6 transfected with pRcpVHLm. A single arrow indicates signals for endogenes, double arrow for exogenes. Panel B sets forth a Northern blot: 1 - original UMRC 6 cells: 2 - UMRC 6 clone 4.

Figure 12: Figure 12 sets forth the sequence of the VHL promoter and surrounding genomic region. This sequence has been deposited in the GenBank database (accession no. U19763). The minimal VHL promoter is underlined. Putative SP1 and AP2 binding sites and upstream termination-polyadenylation site are shown in frame. Horizontal arrows show the start of transcription. Restriction sites for some GC-specific rare cutters are indicated. Position of the 5' end of the group 7 cDNA is shown as vertical arrow. The putative upstream splice acceptor site is double underlined. The first AUG codon in VHL mRNA is shown in a black box.

Figure 13: Figure 13 sets forth the nucleic acid sequences of the partial intron sequences of the VHL disease gene. The upper case letters depict the exon sequences and the lower case letters depict the intron sequences.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the VHL disease gene, its corresponding cDNA and primers corresponding to the VHL wild-type gene sequence. Recently, the region of human chromosome 3 containing the VHL disease gene has been cloned by genomic walking with yeast artificial chromosomes (YACS) and the cloned DNA recovered with cosmids from a chromosome 3 specific library. The phage 191 which contains the VHL disease gene was deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 on May 13, 1993 and has been granted ATCC deposit number 69311. This VHL gene represents the wild-type VHL gene where wild-type means the gene not causing VHL disease or other disease associated with the VHL gene.

The present invention is also directed to a cDNA corresponding to the VHL gene. This cDNA sequence, designated g7, is set forth below as SEQ ID NO: 1 and was deposited with the American Type Culture Collection on May 13, 1993, and has been granted ATCC deposit number 69312. This cDNA also has GenBank accession No. L15409.

```
CCTCGCCTCC GTTACAACAG CCTACGGTGC TGGAGGATCC TTCTGCGCAC   50
GCGCACAGCC TCCGGCCGGC TATTTCCGCG AGCGCGTTCC ATCCTCTACC  100
GAGCGCGCGC GAAGACTACG GAGGTCGACT CGGGAGCGCG CACGCAGCTC  150
CGCCCCGCGT CCGACCCGCG GATCCCGCGG CGTCCGGCCC GGGTGGTCTG  200
GATCGCGGAG GGAATGCCCC GGAGGGCGGA GAACTGGGAC GAGGCCGAGG  250
TAGGCGCGGA GGAGGCAGGC GTCGAAGAGT ACGGCCCTGA AGAAGACGGC  300
GGGGAGGAGT CGGGCGCCGA GGAGTCCGGC CCGGAAGAGT CCGGCCCGGA  350
GGAACTGGGC GCCGAGGAGG AGATGGAGGC CGGGCGGCCG CGGCCCGTGC  400
TGCGCTCGGT GAACTCGCGC GAGCCCTCCC AGGTCATCTT CTGCAATCGC  450
AGTCCGCGCG TCGTGCTGCC CGTATGGCTC AACTTCGACG GCGAGCCGCA  500
GCCCTACCCA ACGCTGCCGC CTGGCACGGG CCGCCGCATC CACAGCTACC  550
GAGGTCACCT TTGGCTCTTC AGAGATGCAG GGACACACGA TGGGCTTCTG  600
GTTAACCAAA CTGAATTATT TGTGCCATCT CTCAATGTTG ACGGACAGCC  650
```

```
TATTTTTGCC AATATCACAC TGCCAGTGTA TACTCTGAAA GAGCGATGCC  700
TCCAGGTTGT CCGGAGCCTA GTCAAGCCTG AGAATTACAG GAGACTGGAC  750
ATCGTCAGGT CGCTCTACGA AGATCTGGAA GACCACCCAA ATGTGCAGAA  800
AGACCTGGAG CGGCTGACAC AGGAGCGCAT TGCACATCAA CGGATGGGAG  850
ATTGAAGATT TCTGTTGAAA CTTACACTGT TTCATCTCAG CTTTTGATGG  900
TACTGATGAG TCTTGATCTA GATACAGGAC TGGTTCCTTC CTTAGTTTCA  950
AAGTGTCTCA TTCTCAGAGT AAAATAGGCA CCATTGCTTA AAAGAAAGTT 1000
AACTGACTTC ACTAGGCATT GTGATGTTTA GGGGCAAACA TCACAAAATG 1050
TAATTTAATG CCTGCCCATT AGAGAAGTAT TTATCAGGAG AAGGTGGTGG 1100
CATTTTTGCT TCCTAGTAAG TCAGGACAGC TTGTATGTAA GGAGGTTTAT 1150
ATAAGTAATT CAGTGGGAAT TGCAGCATAT CGTTTAATTT TAAGAAGGCA 1200
TTGGCATCTG CTTTTAATGG ATGTATAATA CATCCATTCT ACATCCGTAG 1250
CGGTTGGTGA CTTGTCTGCC TCCTGCTTTG GGAAGACTGA GGCATCCGTG 1300
AGGCAGGGAC AAGTCTTTCT CCTCTTTGAG ACCCCAGTGC CTGCACATCA 1350
TGAGCCTTCA GTCAGGGTTT CTCAGAGGAA CAAACCAGGG GACACTTTGT 1400
TAGAAAGTGC TTAGAGGTTC TGCCTCTATT TTTGTTGGGG GGTGGGAGAG 1450
GGGACCTTAA AATGTGTACA GTGAACAAAT GTCTTAAAGG GAATCATTTT 1500
TGTAGGAAGC ATTTTTTATA ATTTTCTAAG TCGTGCACTT TCTCGGTCCA 1550
CTCTTGTTGA AGTGCTGTTT TATTACTGTT TCTAAACTAG GATTGACATT 1600
CTACAGTTGT GATAATAGCA TTTTTGTAAC TTGCCATCCG CACAGAAAAT 1650
ACGAGAAAAT CTGCATGTTT GATTATAGTA TTAATGGACA AATAAGTTTT 1700
TGCTAAATGT GAGTATTTCT GTTCCTTTTT GTAAATATGT GACATTCCTG 1750
ATTGATTTGG GTTTTTTTGT TGTTGTTGTT TTGTTTTGTT TTGTTTTTTT 1800
GGGATGGAGG GAATTC                                      1816
```

The abbreviations used for the nucleotides are those standardly used in the art.

The deduced amino acid sequence of the g7 cDNA is shown as SEQ ID NO: 2 below and starts at nucleotide 1 of SEQ ID NO:1 and extends 851 nucleotides.

```
Pro Arg Leu Arg Tyr Asn Ser Leu Arg Cys Trp Arg Ile Leu Leu
                5                   10                  15
Arg Thr Arg Thr Ala Ser Gly Arg Leu Phe Pro Arg Ala Arg Ser
                20                  25                  30
Ile Leu Tyr Arg Ala Arg Ala Lys Thr Thr Glu Val Asp Ser Gly
                35                  40                  45
Ala Arg Thr Gln Leu Arg Pro Ala Ser Asp Pro Arg Ile Pro Arg
                50                  55                  60
```

Arg	Pro	Ala	Arg	Val	Val	Trp	Ile	Ala	Glu	Gly	Met	Pro	Arg	Arg
				65					70					75
Ala	Glu	Asn	Trp	Asp	Glu	Ala	Glu	Val	Gly	Ala	Glu	Glu	Ala	Gly
				80					85					90
Val	Glu	Glu	Tyr	Gly	Pro	Glu	Glu	Asp	Gly	Gly	Glu	Glu	Ser	Gly
				95					100					105
Ala	Glu	Glu	Ser	Gly	Pro	Glu	Glu	Ser	Gly	Pro	Glu	Glu	Leu	Gly
				110					115					120
Ala	Glu	Glu	Glu	Met	Glu	Ala	Gly	Arg	Pro	Arg	Pro	Val	Leu	Arg
				125					130					135
Ser	Val	Asn	Ser	Arg	Glu	Pro	Ser	Gln	Val	Ile	Phe	Cys	Asn	Arg
				140					145					150
Ser	Pro	Arg	Val	Val	Leu	Pro	Val	Trp	Leu	Asn	Phe	Asp	Gly	Glu
				155					160					165
Pro	Gln	Pro	Tyr	Pro	Thr	Leu	Pro	Pro	Gly	Thr	Gly	Arg	Arg	Ile
				170					175					180
His	Ser	Tyr	Arg	Gly	His	Leu	Trp	Leu	Phe	Arg	Asp	Ala	Gly	Thr
				185					190					195
His	Asp	Gly	Leu	Leu	Val	Asn	Gln	Thr	Glu	Leu	Phe	Val	Pro	Ser
				200					205					210
Leu	Asn	Val	Asp	Gly	Gln	Pro	Ile	Phe	Ala	Asn	Ile	Thr	Leu	Pro
				215					220					225
Val	Tyr	Thr	Leu	Lys	Glu	Arg	Cys	Leu	Gln	Val	Val	Arg	Ser	Leu
				230					235					240
Val	Lys	Pro	Glu	Asn	Tyr	Arg	Arg	Leu	Asp	Ile	Val	Arg	Ser	Leu
				245					250					255
Tyr	Glu	Asp	Leu	Glu	Asp	His	Pro	Asn	Val	Gln	Lys	Asp	Leu	Glu
				260					265					270
Arg	Leu	Thr	Gln	Glu	Arg	Ile	Ala	His	Gln	Arg	Met	Gly	Asp	
				275					280					

The present invention is also directed to intron sequences of the wild-type VHL disease gene. These intron sequences are set forth below as SEQ. ID. NO: 3, SEQ. ID. NO: 4, and SEQ. ID. NO: 5. The lower case letters represent the intron sequences, and the upper case letters represent the surrounding exon sequences.

SEQ. ID. NO: 3

5'-TACCCAACG CTGCCGCCTG GCACGGGCCG CCGCATCCAC AGCTACCGAG gtacgggccc ggcgcttagg cccgacccag caggacgata gcacggtcta agcccctcta ccgccccggg gtccattcag acggggaact aggcccttg aggcaggaca catccagggt -3'

SEQ. ID. NO: 4

5'-ctcctgacct ctatgatccg cctgcctcgg cctccaaagt gctgggatta caggtgtggg ccaccgtgcc cagccaccgg tGTGGCTCtt taacaacctt tgcttgtccc gatagGTCAC CTTTGGCTCT TCAGAGATGC AGGGACACAC GATGGGCTTC TGGTTAACCA AACTGAATTA TTTGTGCCAT CTCTCAATGT TGACGGACAG CCTATTTTTG CCAATATCAC ACTGCCAGgt actgacgttt tactttttaa aaagataagg ttgttgtggt aagtacagga tagaccactt gaaaaattaa gcccagttct caatttttgc ctgatgtcag gcacggtatc caatcttttt gtatcctatt ctctaccata aataaaatgg aagtgatgat ttt -3'

SEQ. ID. NO: 5

5'- ctacagaagg catgaacacc atgaagtgtc catagggggcc acagcataca cactgccaca tacatgcact cacttttttt ctttaaccta aaagtgaaga tccatcagta gtacaggtag ttgttggcaa aagcctcttg ttcgttcctt gtactgagac cctagtctgc cactgaggat ttggtttttg ccc - 3'

The present invention further provides for the following nucleic acid promoter sequence of the wild-type VHL disease gene, designated SEQ. ID. NO: 6:

```
-468  AGAGGCCAAG GCAGGAGGAT CACTTGAACC CAGGAGTTCG
              SP1
-428  AGACCAGCCT AGGCAACATA GCGAGACTCC GTTTCAAACA
         term                          BssHII
-388  ACAAATAAAA ATAATTAGTC GGGCATGGTG GTGCGCGCCT
                       SP1
-348  ACAGTACCAA CTACTCGGGA GGCTGAGGCG AGACGATCGC
-308  TTGAGCCAGG GAGGTCAAGG CTGCAGTGAG CCAAGCTCGC
                   SmaI AP-2                Tth 111 I
-268  GCCACTGCAC TCCAGCCCGG GCGACAGAGT GAGACCCTGT
-228  CTCCAAAAAA AAAAAAAAAC ACCAAACCTT AGAGGGGTGA
-188  AAAAAAATTT TATAGTGGAA ATACAGTAAC GAGTTGGCCT
-148  AGCCTCGCCT CCGTTACAAC AGCCTACGGT GCTGGAGGAT
                                  EagI
-108  CCTTCTGCGC ACGCGCACAG CCTCCGGCCG GCTATTTCCG
                              BssHII BssHII
-68   CGAGCGCGTT CCATCCTCTA CCGAGCGCGC GCGAAGACTA
         SalI       BssHII →      -1   SP1/AP2
-28   CGGAGGTCGA CTCGGGAGCG CGCACGCAGC TCCGCCCCGC
        →SacII      SacII           SmaI
-13   GTCCGACCCG CGGATCCCGC GGCGTCCGGC CCGGGTGGTC
                              SP1/AP2
-53   TGGATCGCGG AGGGAATGCC CCGGAGGGCG GAGAACTGGG
-93   ACGAGGCCGA GGTAGGCGCG GAGGAGGCAG GCGTCGAAGA
                                            NarI
-133  GTACGGCCCT GAAGAAGACG GCGGGGAGGA GTCGGGCGCC
-173  GAGGAGTCCG GCCCGGAAGA GTC
```

Variations are contemplated in the cDNA sequence shown in SEQ. ID. NO: 1 which will result in a DNA sequence that is capable of directing production of analogs of the VHL protein shown in SEQ. ID. NO: 2. It should be noted that the DNA sequences set forth herein represent preferred embodiments of the present invention. Due to the degeneracy of the genetic code, it is to be understood that numerous choices of nucleotides may be made that will lead to a DNA sequence capable of directing production of the instant VHL protein or its analogs. As such, DNA sequences which are functionally equivalent to the sequences set forth herein or which are functionally equivalent to sequences that would direct production of analogs of the VHL protein produced pursuant to the amino acid sequence set forth above, are intended to be encompassed within the present invention.

The term analog includes any polypeptide having an

amino acid residue sequence substantially identical to a sequence specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the function of the VHL protein as described herein. Examples of conservative substitutions include the substitution of non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

The phrase "conservative substitution" also includes the use of a chemically derivatized residue in place of a non-derivatized residue provided that the resulting protein or polypeptide displays the requisite functional activity.

"Chemical derivative" refers to a VHL protein or polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Examples of such derivatized molecules include, but are not limited to, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as chemical derivatives are those proteins or peptides which contain one or more naturally-occurring amino acid derivatives of the twenty standard amino acids. For example: 4-hydroxyproline may be substituted for proline;

5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine. A VHL protein or polypeptide of the present invention also includes any protein or polypeptide having one or more additions and/or deletions or residues relative to the sequence of a polypeptide whose sequence is shown herein, so long as the requisite activity is maintained.

The present invention also relates to methods for detecting carriers of the VHL gene.

It is understood by one skilled in the art that the methods for detection disclosed in the present invention can be used prenatally to screen a fetus or presymptomatically to screen a subject at risk through his/her family history. In addition, these methods can be used to determine the involvement of the VHL gene in other human malignancies such as sporadic renal cancer, uterine cancer, breast cancer, testicular cancer, bladder cancer, pancreatic cancer, ovarian cancer and lung cancer.

Specifically, the methods of the present invention may be used to detect familial types of renal cell carcinoma. Examples of familial types of renal cell carcinoma include, but are not limited to, hereditary, nonpappillary renal cell carcinoma; VHL disease; and hereditary papillary RCC.

Additionally, the methods of the present invention may be used to detect sporadic, noninherited malignancies, such as, for example, renal cell carcinoma.

In one embodiment of the invention, the method for detecting carriers of the VHL gene comprises analyzing the DNA of a subject for mutations of the VHL gene associated with VHL disease, or diseases related thereto.

For purposes of the present invention, subject means a mammal and mutation means inversion, translocation, insertion, deletion or point mutation of the VHL gene.

For analysis of the DNA, a biological specimen is obtained from the subject. Examples of biological specimens that may be analyzed by the methods of the present invention include, but are not limited to, tissue biopsies, whole blood, serum, urine, feces, cerebrospinal fluid or other samples normally tested in the diagnosis of disease. Preferred biological specimens are whole blood or urine.

Although it is not always required, it is preferable to at least partially purify DNA from the biological specimen prior to analysis. For example, after disruption of cells in the specimen, nucleic acid can be extracted from contaminating cell debris and other protein substances by extraction of the sample with phenol. In phenol extraction, the aqueous sample is mixed with an approximately equal volume of redistilled phenol and centrifuged to separate the two phases. The aqueous phase containing the nucleic acid is removed and precipitated with ethanol to yield nucleic acid free of phenol. Alternatively, DNA can be purified from the biological sample according to Sidransky, D. et al. (Science (1992) 256:102-105; Science (1991) 252:706) or by the method of Glenn, et al. (Glenn, G.M. et al. JAMA (1992) 267:1226-1231). The DNA to be analyzed can be either single- or double-stranded.

Methods for analyzing the DNA for mutations in the VHL gene include Southern blotting after digestion with the appropriate restriction enzymes (restriction fragment length polymorphism, RFLP) (Botstein, D. Amer. J. Hum. Genet. (1980) 69:201-205), denaturing gradient electrophoresis technique (Myers, R.M., Nature (1985) 313:495-498), oligonucleotide hybridization (Conner, R. et al., EMBO J. (1984) 3:13321-1326), RNase digestion of a duplex between a probe RNA and the target DNA (Winter, E. et al., Proc. Natl. Acad. Sci. U.S.A. (1985) 82:7575-7579), polymerase chain reaction (PCR) (Saiki, P.K. et al., Science (1988) 239:487-491; U.S. Patents 4,683,195 and 4,683,202), ligase chain

reaction (LCR) (European Patent Application Nos. 0,320,308 and 0,439,182), and PCR-single stranded conformation analysis (PCR-SSCP) (Orita, M. et al., Genomics (1989) 5:874-879; Dean, M. et al. Cell (1990) 61:863-871). In one preferred embodiment, DNA is analyzed by Southern analysis.

The DNA to be analyzed via Southern analysis is digested with one or more restriction enzymes. The restriction enzymes to be used in the present invention are those enzymes for whom the presence or absence of their recognition site is linked to a disease, including, but not limited to, VHL disease and sporadic renal carcinoma. Preferred restriction enzymes include EcoRI, HindIII, PstI, DraI, BamHI, BglI, BglII, and PvuII. Following restriction digestion, resultant DNA fragments are separated by gel electrophoresis and the fragments are detected by hybridization with a labelled nucleic acid probe (Southern, E.M. J. Mol. Biol. (1975) 98:503-517).

The nucleic acid sequence used as a probe in Southern analysis can be labeled in single-stranded or double-stranded form. Labelling of the nucleic acid sequence can be carried out by techniques known to one skilled in the art. Such labelling techniques can include radiolabels and enzymes (Sambrook, J. et al. (1989) in "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Press, Plainview, New York). In addition, there are known non-radioactive techniques for signal amplification including methods for attaching chemical moieties to pyrimidine and purine rings (Dale, R.N.K. et al. (1973) Proc. Natl. Acad. Sci., 70:2238-2242; Heck, R.F. 1968) S. Am. Chem. Soc., 90:5518-5523), methods which allow detection by chemiluminescence (Barton, S.K. et al. (1992) J. Am. Chem. Soc., 114:8736-8740) and methods utilizing biotinylated nucleic acid probes (Johnson, T. K. et al. (1983) Anal. Biochem., 133:126-131; Erickson, P.F. et al. (1982) J. of Immunology Methods, 51:241-249; Matthaei, F.S. et al. (1986) Anal. Biochem., 157:123-128) and methods which

allow detection by fluorescence using commercially available products. The size of the probe can range from about 200 nucleotides to about several kilobases. A preferred probe size is about 500 to about 2000 nucleotides. Each of the nucleic acid sequences used as a probe in Southern analysis is substantially homologous to the corresponding portion of the cDNA sequence shown in SEQ ID NO: 1. By "substantially homologous" is meant a level of homology between the nucleic acid sequence used as a probe and the corresponding sequences shown in SEQ. ID. NO: 1 and SEQ. ID. NOS: 3-6. Preferably, the level of homology is in excess of 70%, most preferably in excess of 80%, with a particularly preferred nucleic acid sequence being in excess of 90% homologous with the sequences shown in SEQ. ID. NO: 1 and SEQ. ID. NOS: 3-6.

Once the separated DNA fragments are hybridized to the labelled nucleic acid probes, the restriction digest pattern can be visualized by autoradiography and examined for the presence or absence of a restriction fragment length polymorphism (RFLP) associated with VHL disease, or diseases related thereto.

In a second preferred embodiment, the DNA is analyzed for mutations in the VHL gene by PCR-SSCP (Orita et al., (1989), Dean et al., (1990)). In this method, each of the pairs of primers selected for use in PCR are designed to hybridize with sequences in the VHL gene which are an appropriate distance apart (at least about 50 nucleotides) in the gene to permit amplification and subsequent detection of mutations in the amplification product. Primer pairs which can specifically hybridize to such VHL gene sequences can be derived from the VHL gene sequence.

In a preferred embodiment, the primers are derived from the cDNA sequences shown in SEQ. ID. NO: 1 and SEQ. ID. NOS: 3-6. Each primer of a pair is a single-stranded oligonucleotide of about 15 to about 50 bases in length which is complementary to a sequence at the 3′ end of one of the strands of a double-stranded target sequence. Each pair

comprises two such primers, one of which is complementary to the 3' end and the other of which is complementary to the other 5' end of the target sequence. The target sequence is generally about 100 to about 300 base pairs long but can be as large as 500-600 base pairs. Optimization of the amplification reaction to obtain sufficiently specific hybridization to the VHL gene is well within the skill in the art and is preferably achieved by adjusting the annealing temperature.

The present invention also provides purified and isolated pairs of primers for use in analysis of DNA for mutations in the VHL disease gene. The nucleic acid sequences of the primers are set forth below as SEQ. ID. NOS: 7-12.

SEQ. ID. NO: 7

ATAGTGGAAA TACAGTAACG AGTTGGCCTA GCCTCGC

SEQ. ID. NO: 8

CCCAGCTGGG TCGGGCCTAA GCGCCGGGCC CGT

SEQ. ID. NO: 9

GTGGCTCTTT AACAACCTTT GCTTGTCCCG ATA

SEQ. ID. NO: 10

CAAGTGGTCT ATCCTGTACT TACCACAACA CCT

SEQ. ID. NO: 11

TGTATACTCT GAAAGAGCGA TGCCTCCAGG T

SEQ. ID. NO: 12

TACCATCAAA AGCTGAGATG AAACAGTGTA AGT

where SEQ ID NO: 7 and SEQ ID NO: 8 represent one pair of primers; SEQ ID NO: 9 and SEQ ID NO: 10 represent a second pair of primers and SEQ ID NO: 11 and SEQ ID NO: 12 represent a third pair of primers.

Additional primers provided by the present invention for use in analysis of DNA for mutations in the VHL disease gene include the following primers, set forth as SEQ. ID. NOS: 13-22:

SEQ. ID. NO: 13

AGTGGAAATA CAGTAACGAG TTGGCCT

SEQ. ID. NO: 14
GAAATACAGT AACGAGTTGG CCTAGC

SEQ. ID. NO: 15
GTCCCAGTTC TCCGCCCTCC GGGGCAT

SEQ. ID. NO: 16
TGGGTCGGGC CTAAGCGCCG GGCCCGT

SEQ. ID. NO: 17
CTTTAACAAC CTTTGCTTGT CCCGATA

SEQ. ID. NO: 18
GTGGCTCTTT AACAACCTTG C

SEQ. ID. NO: 19
GTCTATCCTG TACTTACCAC AACACCT

SEQ. ID. NO: 20
CCTGTACTTA CCACAACACC TTAT

SEQ. ID. NO: 21
CTGAGACCCT AGTCTGCCAC TGAGGAT

SEQ. ID. NO: 22
TTCCTTGTAC TGAGACCCTA GT

SEQ. ID. NO: 23
GGAAATACAGT AACGAGTTGG CCT

SEQ. ID. NO: 24
GGAAATACAG TAACGAGTTG GCCTAGC

SEQ. ID. NO: 25
ACGGGCCCGG CGCTTAGGCC CGACCCA

SEQ. ID. NO: 26
ACGGGCCCGG CGCTTAGGCC CGACCCAGCA GG

SEQ. ID. NO: 27
GTGGCTCTTT AACAACCTTT GCTTGTCCCG ATA

SEQ. ID NO: 28
CTTTAACAAC CTTTGC

SEQ. ID NO: 29
GATAAGGTTG TTGTGGTAAG TACAGGA

SEQ. ID NO: 30
AGGTTGTTGT GGTAAGTACA GGATAGC

SEQ. ID. NO: 31
CTCCTTGTAC TGAGACCCTA GT

SEQ. ID. NO: 32

GTGAGACCCT AGTCTGCCAC TGAGGAT

Examples of primers useful in the present invention which may be used to hybridize to mutant forms of the VHL gene include, but are not limited to, primers that possess the following mutated sequences:

(1) GAGGTCAC (SEQ. ID. NO. 33)

A mutation from the nucleotide sequence GATAGGTCAC to GAGGTCAC in the VHL gene results in the loss of the exon 2 splice acceptor and the loss of expression of exon 2.

(2) GATTGGTCAC (SEQ. ID. NO. 34)

A mutation from the nucleotide sequence GATAGGTCAC to GATTGGTCAC in the VHL gene results in the loss of the exon 2 splice acceptor.

(3) A mutation from G to A at nucleotide 676 of SEQ. ID. NO: 1 and an eight nucleotide deletion of GTACTGAC.

A VHL gene possessing these mutations results in the loss of the exon 2 splice donor.

The primers of this invention can be synthesized using any of the known methods of oligonucleotide synthesis (e.g., the phosphodiester method of Agarwal et al. 1972. Agnew. Chem. Int. Ed. Engl. 11:451, the phosphotriester method of Hsiung et al. 1979. Nucleic Acids Res. 6:1371, or the automated diethylphosphoramidite method of Beuacage et al. 1981. Tetrahedron Letters 22:1859-1862), or they can be isolated fragments of naturally occurring or cloned DNA. In addition, those skilled in the art would be aware that oligonucleotides can be synthesized by automated instruments sold by a variety of manufacturers or can be commercially custom ordered and prepared. In one embodiment, the primers can be derivatized to include a detectable label suitable for detecting and/or identifying the primer extension products (e.g., biotin, avidin, or radiolabeled dNTP's), or with a substance which aids in the isolation of the products of amplification (e.g. biotin or avidin). In a preferred embodiment, SEQ. ID. NO: 7 through SEQ. ID. NO: 34 are

synthetic oligonucleotides.

In an alternative embodiment, primer pairs can be selected to hybridize to mutant forms of the VHL gene. The selected primer pairs will hybridize sufficiently specifically to the mutated gene sequences such that non-specific hybridization to VHL gene sequences will not prevent identification of the amplification product of the mutant gene sequence. Primer pairs which hybridize to mutations in the VHL gene sequence can be used to amplify specific mutant gene sequences present in the DNA of a biological sample.

The amplification products of PCR can be detected either directly or indirectly. In the PCR-SSCP method, direct detection of the amplification products is carried out via labelling of primer pairs. Labels suitable for labelling the primers of the present invention are known to one skilled in the art and include radioactive labels, biotin, avidin, enzymes and fluorescent molecules. The derived labels can be incorporated into the primers prior to performing the amplification reaction. A preferred labelling procedure utilizes radiolabeled ATP and T4 polynucleotide kinase (Sambrook, J. et al. (1989) in "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Press, Plainview, NY). Alternatively, the desired label can be incorporated into the primer extension products during the amplification reaction in the form of one or more labelled dNTPs. In the present invention, the labelled amplified PCR products can be analyzed for mutations of the VHL gene associated with VHL disease gene, or diseases related thereto, via separating the PCR products by denaturing polyacrylamide gel electrophoresis or via direct sequencing of the PCR-products.

In yet another embodiment, unlabelled amplification products can be analyzed for mutations in the VHL gene via hybridization with nucleic acid probes radioactively labelled or labelled with biotin, in Southern

blots or dot blots. Nucleic acid probes useful in the embodiment are those described previously for Southern analysis.

In a second embodiment, the method for detecting carriers of the VHL gene comprises analyzing the RNA of a subject for mutations or alterations in VHL-specific mRNA associated with VHL disease and diseases related thereto, including, but not limited to, sporadic renal cancer, uterine cancer, breast cancer, testicular cancer, bladder cancer, pancreatic cancer, ovarian cancer and lung cancer.

For the analysis of RNA by this method, RNA derived from blood or a tumor biopsy sample is obtained from said subject where said tumors include, but are not limited to, tumors of the eye, brain, liver, kidney, pancreas, and pheochromocytomas.

The RNA to be analyzed can be isolated from blood or tumor biopsy samples as whole cell RNA or as poly(A)$^+$ RNA. Whole cell RNA can be isolated by methods known to those skilled in the art. Such methods include extraction of RNA by differential precipitation (Birnbiom, H.C. (1988) Nucleic Acids Res., 16:1487-1497), extraction of RNA by organic solvents (Chomczynski, P. et al. (1987) Anal. Biochem., 162:156-159) and extraction of RNA with strong denaturants (Chirgwin, J.M. et al. (1979) Biochemistry, 18:5294-5299). Poly(A)$^+$ RNA can be selected from whole cell RNA by affinity chromatography on oligo-d(T) columns (Aviv, H. et al. (1972) Proc. Natl. Acad. Sci., 69:1408-1412). A preferred method of isolating RNA is extraction of whole cell RNA by acid-phenol (Chomczynski et al. 1987).

The methods for analyzing the RNA for alterations in the pattern or level of VHL specific mRNA expression linked to VHL disease and diseases related thereto, include Northern blotting (Alwine, J.C. et al. (1977) Proc. Natl. Acad. Sci., 74:5350-5354), dot and slot hybridization (Kafatos, F.C. et al. (1979) Nucleic Acids Res., 7:1541-1522), filter hybridization (Hollander, M.C. et al. (1990)

Biotechniques; 9:174-179), RNase protection (Sambrook, J. et al. (1989) in "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Press, Plainview, NY) and reverse-transcription polymerase chain reaction (RT-PCR) (Watson, J.D. et al. (1992) in "Recombinant DNA" Second Edition, W.H. Freeman and Company, New York). One preferred method is Northern blotting.

The nucleic acid sequence used as a probe for detecting VHL-specific mRNA expression is substantially homologous to SEQ. ID. NO: 1. By "substantially homologous" is meant a level of homology between the nucleic acid sequence and the cDNA sequence of SEQ. ID. NO: 1. Preferably, the level of homology is in excess of 70%, more preferably in excess on 80%, with a particularly preferred nucleic acid sequence being in excess of 90% homologous with the cDNA sequence shown in SEQ. ID. NO: 1.

A most preferred method is reverse transcription-polymerase chain reaction (RT-PCR) where the primers used to amplify the cDNA produced via reverse transcription of RNA are derived from the cDNA sequence shown in SEQ. ID. NO: 1. These primers can be labelled as described earlier and the RT-PCR products can be analyzed for mutations of the VHL gene associated with VHL disease, or diseases related thereto, via denaturing polyacrylamide gel electrophoresis of the RT-PCR products or via direct sequencing of the RT-PCR products.

In a third embodiment, the method for detecting carriers of the VHL gene comprises analyzing the DNA of a subject for mutations or alterations in VHL-specific DNA associated with VHL disease, or diseases related thereto, such as sporadic renal cancer, uterine cancer, breast cancer, testicular cancer, bladder cancer, pancreatic cancer, ovarian cancer and lung cancer.

The present invention also encompasses recombinant proteins derived from the cDNA shown in SEQ. ID. NO: 1 and antibodies directed to said proteins (called VHL proteins).

Recombinant VHL proteins can be produced by recombinant DNA methodology known to one skilled in the art. For example, a nucleic acid sequence capable of encoding a protein comprising all or part of the amino acid sequence shown in SEQ. ID. NO: 2 can be cloned into a vector capable of being transferred into, and replicated in, a host organism. A suitable nucleic acid sequence for the purpose of this invention are the sequences shown in SEQ. ID. NO: 1 and SEQ. ID. NOS: 3-6. Suitable expression vectors include, but are not limited to, vaccinia virus vectors, baculovirus vectors, and E. coli pTRCHIS (Invitrogen Co. San Diego). The recombinant expression vector produced by inserting a nucleic acid sequence capable of directing synthesis of VHL protein in a suitable expression vector can be transfected into E. coli or into suitable eukaryotic cell systems by methods known to one skilled in the art.

Cells containing the expressed recombinant VHL protein, cell lysate from cells transfected with a recombinant expression vector or a culture supernatant containing the expressed VHL protein can be used as an immunogen to elicit production of anti-VHL antibodies in a mammal. Alternatively, one can generate synthetic peptides for use as immunogens from the amino acid sequence shown in SEQ. ID. NO: 2.

Preferred synthetic peptide sequences for use as immunogens are shown below:

SEQ ID NO. 35:

Glu Glu Tyr Gly Pro Glu Glu Asp Gly Gly Glu Glu Ser Gly

SEQ ID NO. 36:

Gly Thr Gly Arg Arg Ile His Ser Tyr Arg Gly His Leu

While it is possible for the immunogen to be administered to the mammal in pure or substantially pure

form, it is preferable to present it as a pharmaceutical composition, formulation or preparation. Suitable mammals for immunization include mice, rabbits and the like. The anti-VHL antibody of the present invention is typically produced by immunizing a mammal with an immunologically effective amount of synthetic peptide of this invention. The preparation of polyclonal or monoclonal antibodies against such a peptide is well known in the art (Standt, et al. (1988) J. Exp. Med. 157:687-704). The anti-VHL peptide antibody molecules induced by immunization of a mammal with the recombinant VHL protein are then collected from the mammal and those immunospecific for the VHL protein are isolated to the extent desired by well known techniques such as, for example, immunochromatography.

In a third embodiment, the method for detecting carriers of the VHL gene comprises:

analyzing the protein of a subject for alterations in VHL protein expression.

For analysis of protein by this method, protein is obtained from biological specimens such as tumor biopsy samples and urine and the like. The protein can be obtained as a crude lysate or it can be further purified by methods known to one skilled in the art (Sambrook, J. et al. (1989) in "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor press, Plainview, NY).

Crude protein lysate can be analyzed for VHL protein by immunoassays using anti-VHL antibody.

Immunoassays of the present invention may be a radioimmunoassay, Western blot assay, immunofluorescent assay, enzyme immunoassay, chemiluminescent assay, immunohistochemical assay and the like. Standard techniques known in the art for ELISA are described in <u>Method in Immunodiagnosis</u>, 2nd Edition, Rose and Bigazzi, eds., John Wiley and Sons, 1980 and Campbell et al., <u>Methods of Immunology</u>, W.A. Benjamin, Inc., 1964, both of which are incorporated herein by reference. Such assays may be a

direct, indirect, competitive, or noncompetitive immunoassay as described in the art. (Oellerich, M. 1984. J. Clin. Chem. Clin. BioChem. 22:895-904).

Detection of the VHL protein anti-VHL antibody complex formed can be accomplished by reaction of the complex with a secondary antibody such as labelled anti-rabbit antibody. The label may be an enzyme which is detected by incubating the complex in the presence of a suitable fluorimetric or colorimetric reagent. Other detectable labels may also be used, such as radiolabels, or colloidal gold, and the like. The labelled VHL protein-anti-VHL antibody complex is then visualized by autoradiography.

The present invention also relates to a method for treating a carrier of the VHL gene in which an expression vector containing a nucleic acid sequence representing the VHL gene is administered to the carrier. Nucleic acid sequences representing the VHL gene are SEQ. ID. NO: 1 and SEQ. ID. NOS: 3-7. Such nucleic acid sequences may be inserted into a suitable expression vector by methods known to those skilled in the art (Example 5). Expression vectors suitable for producing high efficiency gene transfer in vivo include retroviral, adenoviral and vaccinia viral vectors.

Expression vectors containing a nucleic acid sequence representing the VHL gene can be administered intravenously, intramuscularly, subcutaneously, intraperitoneally or orally. A preferred route of administration is intravenously.

The invention also provides a diagnostic kit for detecting carriers of the VHL gene. This diagnostic kit comprises purified and isolated nucleic acid sequences according to SEQ ID. NO: 7 through SEQ ID NO: 34, said sequences useful as PCR primers in analyzing DNA for the presence of mutations of the VHL gene linked to VHL disease, or diseases related thereto.

The invention also provides a diagnostic kit for

detecting regulatory defects of the VHL gene. This diagnostic kit comprises purified and isolated nucleic acid sequences according to SEQ. ID. NO: 7 through SEQ. ID. NO: 34, said sequences useful as PCR primers in analyzing DNA for mutations of the VHL gene linked to VHL disease and diseases related thereto, including, but not limited to, sporadic renal cancer, lung cancer, uterine cancer, breast cancer, testicular cancer, ovarian cancer, adrenal tumors, brain tumors, lung tumors or other cancers.

The nucleic acid sequences of the present invention according to SEQ. ID. NO: 7 through SEQ. ID. NO: 34 are useful in the detection of hereditary and sporadic kidney cancers by the detection of abnormalities of the VHL gene in biological samples using the primers of the present invention.

The present invention further provides a method of preventing or treating regulatory defects linked to VHL disease. Specifically, the present invention provides a method of treating or preventing cancer in a subject by contacting the cancer with an amount of the VHL gene of the present invention effective to treat the cancer. This method comprises administration of the VHL gene in an amount effective to prevent or treat regulatory defects associated with VHL disease and diseases related thereto, including, but not limited to, sporadic renal cancer, lung cancer, uterine cancer, breast cancer, testicular cancer and ovarian cancer.

In one embodiment of the invention, the VHL gene sequence or analog thereof is administered in a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier encompasses any of the standard pharmaceutical carriers such as sterile solution, tablets, coated tablets and capsules. Such carriers may typically contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stensic acid, talc, vegetable fats or olis, gums, glycols, or other known excipients. Such

carriers may also include flavor and color additives and other ingredients.

Types of cancer that may be treated using the VHL sequences or proteins of the present invention include, but are not limited to, VHL disease and diseases related thereto, including, but not limited to, sporadic renal cancer, lung cancer, uterine cancer, breast cancer, testicular cancer, ovarian cancer, adrenal tumors, brain tumors, lung tumors or other cancers.

Specific carcinomas which may be treated using the VHL sequences or proteins of the present invention include, but are not limited to, renal cell carcinoma, pheochromocytoma, retinal angioma, hemangioblastoma, pancreatic cysts, pancreatic tumors and epididymal cystadenoma.

Any articles or patents referenced herein are incorporated by reference. The following examples illustrate various aspects of the invention but are in no way intended to limit the scope thereof.

MATERIALS

The subjects analyzed in the following examples were kindred identified by ophthalmologists, urologists, medical geneticists and neurosurgeons in the United States, Europe, and Canada. The members of the families resided in Louisiana, Tennessee, Mississippi, Virginia, Pennsylvania, New York, Michigan, Quebec, Nova Scotia, United Kingdom, and the Netherlands. Medical records of each family member known to be affected were reviewed. Asymptomatic family members and family members in whom there was uncertainty about the diagnosis were examined after informed consent for occult evidence of the illness at the Clinical Center of the National Institutes of Health. The examination consisted of a history and physical examination of the scrotum. An asymptomatic member of a VHL family was considered to be affected if one or more of the following disease

manifestations were detected: retinal angioma(s), spinal or cerebellar hemangioblastoma(s), pheochromocytoma(s), multiple pancreatic cysts, and multiple bilateral renal cysts accompanied by renal cell carcinoma. Disease diagnosis was made without knowledge of restriction fragment length polymorphism (RFLP) status.

Restriction enzymes were from Bethesda Research Laboratory (BRL) (Bethesda, MD), New England Biolabs (Beverly, MA) and Boehringer Mannheim (Indianapolis, IN) and were used as recommended by the manufacturers. δ-^{32}PdCTP (~3000iu/mmol) was from Amersham (Arlington Heights, IL). The various human tissue polyadenylated RNAs used in Northern blotting were purchased from Clontech (Palo Alto, CA) as was the adult kidney double-stranded complementary DNA sample. PCR and RT-PCR bits were from Perkin Elmer/Cetus (Norwalk, CT); deoxynucleotide triphosphates and flourescently labelled dideoxynucleotides were from Applied Biosystems, Inc. (Foster City, CA). Nylon membranes were purchased from MSI, Inc. (Westlore, MA).

METHODS

Southern and Northern blottings, filter hybridization and probe labelling were carried out using random priming and were otherwise performed by standard protocols (Sambrook, J. et al. (1989)). DNA inserts were purified following the GeneClean (Bio 101) (BioRad, Richmond, CA) protocol and used for subcloning or labelling. Oligonucleotides used as primers in PCR or RT-PCR or for sequencing were synthesized on the Applied Biosystems, Inc. Model 392 DNA/RNA synthesizer, according to the manufacturers recommendations. Pulse field goal electrophoresis was carried out using CHEF-DRII or CHEF mapper XA systems as described by the manufacturer (BioRad) under conditions optimal for obtaining the desired resolution.

PCR - The PCR was performed in a 50 ul reaction

volume in a mixture containing 1uM of each primer, 250uM of each deoxynucleotide triphosphate, 5ul of 10X PCR buffer (500MM KCl; 120MM Tris-HCl, pH 8.0; 1.5MM $MgCl_2$; and 0.1% gelatin) and 1.25 units of AmpTaq (Cetus) DNA polymerase, in a first generation automated thermal cycler (Perkin-Elmer/Cetus). The PCR conditions consisted of 40 cycles of denaturation for one minute at 94°C, annealing for one minute at specified temperatures (55-65°C) and extension for 4 minutes at 72°C followed by 7 minutes of final extension of 72°C.

RNA Preparation and Northern Blotting - Total cellular RNA was isolated by extraction of lymphoblastoid cell lines of affected VHL patients or kidney tissues in guanidine thiocyanate followed by centrifugation through a 5.7 M CsCl cushion according to standard protocols (Sambrook, J. et al. (1989)). RNA samples were separated by electrophoresis in 1% agarose gels containing 2.2M formaldehyde, transferred to nylon membranes and hybridized to g7 cDNA probe (Sambrook, J. et al. (1989)).

RT-PCR - About 5 ug of total cellular RNA was isolated by extraction of lymphoblastoid cell lines or kidney tissues of VHL patients or 2.5 ng of normal adult kidney double-stranded complementary DNA samples were analyzed for expression using RT-PCR kit from Perkin-Elmer/Cetus. The primers were derived from the g7 cDNA sequence shown in SEQ. ID. NO: 1 and the reactions were run using various annealing temperatures. The reaction products were analyzed by gel electrophoresis and Southern blotting (Sambrook, J. et al (1989)).

Cell Culture - The 293 cells (Graham, et al. 1977) and UMRC 6 cells (Grossman, et al. 1995) were grown in DMEM medium supplemented with 10% fetal bovine serium (Life Technologies Inc., NY) penicillin (25 000 U/I) and streptomycin (25,000 μg^{-1}) with 8% CO_2.

Isolation of RNA for Identification of Promoter Region - Total RNA from cell cultures was isolated using

Ultraspec II RNA isolation system (Biotex, TX). Poly(A)$^+$ RNA was purified twice on oligo-dT Cellulose (Stratagene, CA).

RNAse H mapping - Ten micrograms of total RNA and 200 ng of VHL-specific antisense oligomer were annealed and RNA was digested with RNAse H essentially as described by Berger (1987). The following oligonucleotides were used; for VHL exon 1 (SEQ. ID. NO. 37): 5'- ACG ACG CGC GGA CTG CGA TTG CAG AAG AT-3': for exon 3 (SEQ. ID. NO. 38): 5'-AGC GAC CTG ACG ATG TCC AGT CTC-3'. After ethanol precipation, RNA was separated in 0.75% agarose-formaldehyde gels (Lehrah, et al., 1977) transferred to nylon membrane and hybridized to the probe.

Mapping of the Transcription Start Site - Transcription start mapping was performed using Ribonuclease Protection Assay Kit (RPA II, Ambion, TX) according to manufacturer instructions. Protected fragments were separated in a standard urea sequencing gel (6% polyacrylamide). The gel was vacuum dried and exposed to X-ray film (Kodak X-OMAT AR). Sequencing ladder was made using control template, primer and reagents from Sequenase Version 2.0 DNA sequencing kit (United States Biochemical, OH).

RNA markers, probes and control sense VHL RNA were obtained by *in vitro* transcription using RNA Maxiscript T3/T7 kit (Ambion, TX) and three groups of templates. The first group (Figure 8A, probes 1, 2, 3 and 4) derived from plasmid pBluescript II S/K (Stratagene, CA) carrying an inserted 892bp EcoRI-NotI genomic fragment, containing the 5' part of VHL exon 1 and 5' flanking genomic region (-647/+245). For generation of probes no. 1, no. 2, no. 3 and no. 4 some parts of the insert were deleted and derivative plasmids were linearized as shown in Figure 8A. The second group of templates was generated by PCR using the primers 5'-CCT CGC CTC CGT TAC AAC A-3' (SEQ. ID. NO. 39) and 5'-GGA TCC TAA TAC GAC TCA CTA TAG GGA GGC GCC CGA CTC CTC CC-3' (SEQ. ID. NO. 40). This PCR fragment contained part of the

genomic EcoRI-NotI sequence (residues - 166/+173) and the promoter of T7 RNA polymerase to make antisense VHL probe. To generate several marker probes, the template was cleaved around presumptive transcription start sites with EagI, BssHII, Alul or BamHI (Figure 8A, probes 5, 6, 7 and 8). These probes were hybridized to probe no. 4 (control sense RNA) and the protected fragments were used as markers on Figure 8C. The third set of templates (RNA Century Marker Template Set) was purchased from Ambion (TX). All templates were blunt ended with Klenow fragment.

Luciferase Plasmid Construction - Presumptive promoter region was amplified by PCR using upstream (sense) primer 5'-CTA TCT AGA GGC CAA GGC AGG AGG ATC-3' (SEQ. ID. NO. 41) and two downstream (antisense) primers: 5'-CAT TCT AGA TTC CCT CCG CGA TCC AGA-3' (SEQ. ID. NO. 42) and 5'-CAT TCT AGA CTC TTC CGG GCC GGA CTC-3'(SEQ. ID. NO. 43). The two PCR fragments contained residues 180-716 and 180-842 of the genomic EcoRI-NotI fragment (respectively residues -468-69 and -468+195 on Figure 12) and XbaI linkers. PCR fragments were digested with XbaI and cloned in both orientations into the NheI site of the pGL-2 enhancer vector (Promega, WI). Series of 3' and 5' deletion constructs were generated using appropriate unique restrictases within the insert and in pGL-2 polylinker (MluI - for 5' deletions and BglII for 3' deletions). The plasmids carrying SV 40 early promoter (in pGL-2 control: Promega) and thymidine kinase promoter (in pTK, Gill, et al., 1994) were used as positive controls.

Transfection and Assays of Luciferase Activity - 293 and UMRC 6 cells were transfected using the lipofectin protocol as described elsewhere (Chang and Brenner, 1988). For each 35mm plate 2μg of the luciferase reporter plasmid, 1μg of pCMVβ (Clontech, CA) and 10μl of Lipofectin (Gibco-BRL) were added. Luciferase and β-galactosidase assays were performed 40h after transfection using luciferase and β-galactosidase assay kits (Promega). The luciferase assay

was performed using a Monolight 2010 luminometer (Analytical Luminescence Laboratory, CA).

Construction of the VHL Minigenes - Expression construct (pRc-HAVHL), which contained VHL reading frame subcloned into pRc CMV vector (Invitrogen, CA), was kindly provided by Dr. William G. Kaelin Jr. (Division of Neoplastic Disease Mechanisms, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA). Group 7 VHL cDNA in pBluescript II KS was described elsewhere (Latif, et al., 1993), 1.4kb NotI fragment from group 7 construct (exons 3, 2 and 3' part of exon 1) was inserted in correct orientation into NotI site of plasmid pNE (pBluescript II SK carrying VHL 5' flanking 892bp EcoRI-NotI genomic fragment, including 5' part of exon 1). The final plasmid (pVHL) was used to generate three expression constructs in which VHL minigene was driven by its own promoter as follows: (1) pRcpVHL: after digestion of pRc-HAVHL with NruI-BstEII, CMV promoter/enhancer and part of the VHL reading frame were removed and substituted by VHL promoter and exon 1 from pVHL (EcoRV-BstEII digest); (2) pRcpVHLm: plasmid pRcpVHL was linearized with BstEII, filled-in with Klenow fragment and religated: (3) pRcpVHL3U: BstEII-XbaI fragment in pRcpVHL was substituted by BstEII-XbaI fragment from pVHL, which contained additional 0.9kb from 3' untranslated region.

Stable Transfection of the UMRC6 Cells - Eighty percent confluent UMRC 6 cells were transfected with 25 μg DNA and 40 μl of lipofectin in 5 ml OPTI-MEM medium (Life Technologies Inc., NY) per 100 mm plate for 12 h and grown in DMEM medium. In a day, 400 μg ml^{-1} of active geneticin was added and resistant colonies were grown for 2 to 3 weeks. Selective media was changed every 3 days.

EXAMPLES

The Examples herein are meant to exemplify the various aspects of carrying out the invention and are not intended to limit the scope of the invention in any way.

Example 1

Isolation of the VHL Disease Gene

The isolation of the VHL disease gene resulted from the use of positional cloning strategies (Latif et al., Cancer Res. (1993) 63:861-867; Trofatter et al., Cell (1993) 72:791-800 and The Huntington's Disease Collaborative Research Group; Cell (1993) 72:971-983) previously used in isolating disease genes and is described in Latif, et al., Science, (1993) 260:1317-1320. Genetic and physical map of the chromosome 3p region encompassing the VHL gene is shown in Figure 1. The VHL locus was positioned on the map (Fig. 1A) by multipoint linkage analysis and meiotic mapping (Tory et al., 1989); the location of selected cross-overs is indicated by crosses.

YAC Library Screening and Analysis of YACs. Copies of the WU and CEPH YAC libraries were obtained from Dr. Craig Chinault (Baylor Institute of Human Genetics, Houston, Texas) and Dr. Daniel Cohen, respectively (centre d' Etude du Polymorphisme Humain, Paris). The WU and CEPH libraries are total human genomic DNA libraries constructed in the PYAC4 vector (Burke, D.T. et al. Science (1987) 236:806-812; Anand, R. et al. Nucleic Acids Res. (1990) 18: 1951-1956). These libraries were screened by sib selection using PCR-based techniques (Greene, E.D. et al., Proc. Natl. Acad Sci. (1990) 87:1213-1217) with primers for the D3S601, D3S587 and D3S18 loci in the VHL region (Figure 1). The sequences of the primers used to positively identify YACs Y52A10, YA101D4, Y132F2 and Y70D2 are shown below as SEQ. ID. NO. 44 thru SEQ. ID. NO. 49:

Locus/ Location	Designation	Sequence
D3S18/3p26	ML-1	CACAAGTGAT GCCTTGTAGC TG SEQ. ID. NO. 44
D3S18/3p26	ML-2	CAGTAGTGTC CTGTATTTAG TG SEQ. ID. NO. 45

D3S601/3p25.3	ML-7	GTTGGCTATG GGTAGAATTG G SEQ. ID. NO. 46
D3S601/3p25.3	ML-8	CAGGGTAGCC TTGATCTAAG T SEQ. ID. NO. 47
D3S587/3p25.2	ML-10	GGAGGTCCTG AGAATATGTG TCC SEQ. ID. NO. 48
D3S587/3p25.2	ML-11	TGTTCAGGCA CACAGTAGAT G SEQ. ID. NO. 49

Screening Chromosome 3 Cosmid Library and Cosmid Contig Assembly. The chromosome 3 cosmid library was constructed as described in Lerman, et al. (Lerman, M.I. et al. Hum. Genet. (1991) 86:567-577). This library was screened by colony hybridization (Sambrook, J. et al. (1989)) using the YAC DNA inserts as probes as described in Baxendale, et al. (Baxendale, S. et al. Nucl. Acids Res. (1991) 19:6651). After labeling with ^{32}P-dCTP, the probes were preassociated with a 1000X excess of sheared human DNA. Cosmid contigs were constructed by finding overlapping bands on Southern blots of EcoRI-digested cosmids using whole cosmids as probes. Gaps in the cosmid contigs were closed by chromosome walking using insert-end fragment probes, which were identified by restriction mapping and hybridization to restricted genomic DNA. These insert-end fragment probes were used for each walk step. Figure 1(c) shows the 160 kb cosmid and phage contig covering the VHL region. The phage T42 was isolated by screening a total genomic phage library with YAC DNA inserts as described above. The phage p191, which contains the VHL gene, was isolated by screening a three-hit P1 phage genomic library (Genome System, Inc. St. Louis, MO) with primers chosen from within an exon of the g7 cDNA sequence shown in SEQ ID NO. 1. The phage p191 was deposited with the ATCC on May 13, 1993.

Example 2

Isolation of a cDNA Corresponding to VHL Disease Gene

Screening cDNA Libraries. A λgt11 teratocarcinoma library (gift of Dr. Maxine Singer, National Cancer Institute) was screened by plaque hybridization (Sambrook, J. et al. (1989)) to 10° filter-immobilized cDNA phage clones at a density of 4 x 10^4 pfu/150-mm filter. Figure 1B shows the position of the g7 cDNA isolated by screening the λgt11 teratocarcinoma cDNA library with a conserved Fkb fragment at the centromeric end of cos11 used as a probe in the screening. The orientation of the g7 cDNA was established by sequencing and restriction mapping to the contig. The beginning of the smallest constitutional deletion is indicated by an asterisk and line. Restriction sites: B, Bam HI; E, Eco RI; N, Not I; Nr, Nru I; M, Mlu I.

cDNA Sequence and Sequence Analysis. The g7 cDNA clone was sub-cloned into the Bluescript KS (+) plasmid (Stratagene, La Jolla, CA). Double-stranded plasmid DNA was used in sequencing reactions performed with Tag Dye Deoxy terminator cycle sequencing kits (Applied Biosystems, Inc.). All sequences were obtained by running the reactions in an ABI 373A automatic sequencing system (Applied Biosystems, Inc.). Initial sequencing was performed with T3 and T7 primers, and "walking" primers were then constructed to continue sequencing. The cDNA clone was sequenced multiple times in one orientation or both orientations. Database searching, sequence editing, sequence assembly, and sequence analysis were carried out with the University of Wisconsin Genetics Computer Group sequence analysis software package, version 7.0 (Devereaux, J. et al. Nucl. Acids Rev. (1984) 12:387-395). The sequence of the g7 cDNA is shown in SEQ ID No. 1. This cDNA was deposited with the ATCC on May 13, 1993. The cDNA sequence revealed an open reading frame (ORF) of 284 amino acids indicating that the rest represents part of the 3' untranslated region of the mRNA. This ORF showed a high

probability score (> 95%) for being a protein coding sequence Fickett, J.W., Nucl. Acids Rev. (1982) 10:5303). Neither the nucleotide nor the predicted amino acid sequences showed any significant homology to genes or proteins in the databases.

Example 3

Detection of g7-Specific mRNA Expression in Target Tissues

RNA Preparation and Northern Blotting Analysis. To identify the VHL gene, the g7 loci was evaluated by analyzing its expression in target tissues.

The expression pattern of the g7 gene was examined by Northern (RNA) blotting. Figure 2A shows a low resolution blot where each lane contains poly A^+ mRNA (2 μg) from: lane 1, fetal brain; lane 2, adult brain; lane 3, fetal kidney; lane 4, adult kidney; lane 5, adult cerebellum; lane 6, adult adrenal; and lane 7, adult prostate while Figure 2B shows a high resolution blot of 1 ug of poly A^+ mRNA from tissues as indicated in Figure 2A. The sizes of the transcripts were determined from the position of the 28*S* and 18*S* rRNA bands of total RNA run on the same gel. Transcripts were observed in all human tissues tested, including brain and kidney, tissues frequently affected in VHL disease. The transcripts were of two distinct sizes, 6 and 6.5 kb, and were expressed in a tissue-specific and developmentally selective manner, i.e. only 6 kb or the 6.5 kb species was expressed in fetal brain and fetal kidney, while both were expressed in adult tissues. The two transcripts may represent alternatively spliced forms of g7 mRNA.

Example 4

Detection of Mutations of the VHL Disease Gene Associated With VHL Disease and Related Diseases

RT-PCR Studies of Gene Expression. In order to detect mutations in constitutional DNA of affected patients in

pedigrees and in new mutation patients, an extensive search for mutations (i.e. small intragenic and nonoverlapping deletions or insertions) which were of the loss -of-function type was conducted in constitutional DNA derived from 221 unrelated VHL patients. Southern blot analysis of genomic DNA isolated from the blood (Sambrook, J. et al. (1989)) of seven patients and then digested with EcoRI is shown in Figure 3A. This blot was probed using the g7 cDNA as probe. This probe has been shown to detect a single invariant 20-22 kb EcoRI fragment in normal DNA, as determined by previous tests on more than 100 unrelated DNA samples provided by Centre d'Etude du Polymorphisme Humain (CEPH). A high incidence (≥ 12%) of aberrant bands was observed with the bands ranging in size from 4 to 25 kb (Figure 3A), and these VHL patients were thus classified as new mutations.

In order to determine that the single aberrant bands originating from the 20-22 kb invariant fragment were deletions or insertions within this fragment or deletions removing the flanking EcoRI sites, Southern blot analysis was conducted with several other restriction enzyme digests besides EcoRI (BamHI, BglI, BglII, DraI, EcoRV, HindIII, PstI, and PvuII). The results of the Southern analysis with a few of these enzymes is shown in Figure 3B. These results demonstrated that the mutations were transmitted with the disease. Figure 3C shows the results of Southern blotting analysis of DNA isolated form a regular VHL family (coded "P") and digested with EcoRI. The results clearly demonstrate transmission of the mutant allele (the aberrant band) in this VHL family.

__Example 5__

__Detection and Mapping of Deletions of the VHL Disease Gene__

To prove the presence of deletions and to map them precisely, subfragments representing regions of the g7 cDNA generated by PCR were used as probes in Southern blotting

analysis of genomic DNA isolated from blood of VHL patients and digested with EcoRI. (Figure 4, where the probes used in each panel are: Panel A, total g7 cDNA; Panel B, nucleotides 3-146 of g7 cDNA; and Panel C, nucleotides 1277-1600 of g7 cDNA). The results unequivocally demonstrated that 18 of the rearrangements were deletions as only part of the cDNA failed to detect the novel band in each patient (Figure 4).

These deletions could then be classified into three groups, as shown in Table 1.

TABLE 1

Deletion analysis of VHL patients with aberrant bands at the VHL locus (detected by g7 cDNA).

Patient Code	Probe : cDNA 5'—>3' residue (s)						Aberrant Band (kb)	Apparent Deletion Size (kb)
	3-146	169-391	291-501	585-940	921-1231	1277-1600		
3567	ND	ND	ND	ND	ND	ND	14	?
3607	ND	ND	ND	ND	ND	ND	12	?
3639	ND	ND	ND	ND	ND	ND	14	?
3648	ND	ND	ND	ND	ND	ND	13	?
3654	ND	ND	ND	ND	ND	ND	14	?
JD	ND	ND	ND	ND	ND	ND	17	?
PEM	ND	ND	ND	ND	ND	ND	15	?
MS	ND	ND	ND	ND	ND	ND	15	?
KA	ND	ND	ND	ND	ND	ND	15	?
3547	D	D	D	ND	ND	ND	23-25	15-18
JM	D	D	D	ND	ND	ND	23-25	15-18
GD	D	D	D	ND	ND	ND	23-25	15-18
3512	ND	ND	ND	ND	D	D	10	11
3516	ND	ND	ND	ND	D	D	10	11
3557	ND	ND	ND	ND	D	D	10	11
3574	ND	ND	ND	ND	D	D	10	11
VIA	ND	ND	ND	ND	D	D	10	11
IC	ND	ND	ND	ND	D	D	10	11
NE	ND	ND	ND	ND	D	D	10	11
EP	ND	ND	ND	ND	D	D	10	11
MO	ND	ND	ND	ND	D	D	10	11
3569	ND	ND	ND	D	D	D	12	9
3667	ND	ND	ND	D	D	D	10	11
3761	ND	ND	ND	D	D	D	4	17
3819	ND	ND	ND	D	D	D	12	9

ND = Not deleted
D = Deleted

The finding of three overlapping deletions within the same cDNA provides strong evidence for the identification of the g7 cDNA as the VHL gene.

Example 6

Detection of Intragenic Deletions or Insertions by PCR-SSCP and RT-PCR

To find intragenic deletions or insertions, genomic DNA isolated from VHL patient lymphoblastoid cell lines (Lymphoblastoid cells were immortalized by transformation with Epstein Barr Virus according to standard protocols (Nilison, K. et al., Adv. Cancer Res. (1982) 37:319-380)) was analyzed for alterations by PCR-single-strand-conformational polymorphism (PCR-SSCP) analysis using primers shown in SEQ. ID. NO. 7 thru SEQ. ID. NO. 12 and RNA isolated from sporadic renal cell carcinoma (RCC) cell lines (Anglard, P. et al. Cancer Res. (1992) 52:348-356) was analyzed by reverse transcription-polymerase chain reaction (RT-PCR). The primers used for RT-PCR of the RCC cell lines are shown as SEQ. ID. NO. 50 thru SEQ. ID. NO. 53:

SEQ. ID. NO. 50
CATCTTCTGC AATCGCAGTC CGCGCGT

SEQ. ID. NO. 51
CAAAAGCTGA GATGAAACAG TGTAAGT

SEQ. ID. NO. 52
GTTTGGTTAA CCAGAAGCCC ATCGT

SEQ. ID. NO. 53
GATGGGCTTC TGGTTAACCA AACT

whose SEQ. ID. NO. 50 and NO. 51 are on pair of primers and SEQ. ID. NO. 52 and SEQ. ID. NO. 53 are a second pair. The results of these analyses are shown in Table 2.

TABLE 2

Germ-line (VHL) and somatic (sporadic RCC) mutations in the VHL candidate gene.

Patients	Mutation	Consequence
VHL family		
"VA"	8 bp (TTGTCCGT) insertion after NT714*	frameshift
"E"	9 bp in-frame deletion (NT456-464)	Three amino acid (153-154) deletion (Arg Val Val)
"CS"	3 bp in-frame deletion (NT434-436)	One amino acid deletion (146, Ile)
Sporadic RCC		
"UOK118"	1 bp deletion (NT737)	frameshift
"UMRC5"	1 bp deletion (NT737)	frameshift
"UMRC6"	10 bp deletion (NT715-724)	frameshift
"A498"	5 bp deletion (NT638-642)	frameshift
"UOK151"	nonsense C → A (NT761) transversion	stop codon

*NT = nucleotide(s).

RCC were chosen because according to Knudson's dictum (Knudson (1971)), sporadic cancers should be associated with mutations in the same loci affected in the hereditary form of the same malignancy. So far aberrant patterns have been identified in five RCC cell lines and proved four of them have been proven to be small (1 to 10 bp) deletions creating frameshift mutations and truncated proteins (TABLE 2). The cell lines UMRC5 and RCC "UOK118" have the same 1 bp deletion at nucleotide 737, amino acid 246, creating 28 new amino acids followed by a stop codon. Incidentally, this deletion creates a new EcoRI site, leading to two aberrant bands on Southern blots (not shown). Line UMRC6 has a 10 bp deletion (nucleotides 715 to 724) creating a frameshift such that 32 new amino acids are present followed by a new stop codon. Finally, line A498 has a 5 bp deletion (nucleotides 638 to 642) leading to a premature stop after new 62 amino acids. In the fifth RCC cell line, UOK151, the change is a nonsense (stop codon) mutation resulting from a C to A transversion at nucleotide 761 (TCG → TAG), creating a truncated protein. These data suggest that the VHL disease gene plays an important role in sporodic kidney cancer. As such, RT-PCR or PCR-SSCP as described in this application can be used as diagnostic methods to distinguish primary kidney tumors from tumors that spread to the kidney from other tissues or organs and to distinguish different histological types of kidney tumors.

In the DNA of the VHL lymphoblastioid cell lines derived from VHL patients, SSCP aberrant patterns segregating with the disease were also detected using primers shown in SEQ. ID. NO. 7 thru SEQ. ID. NO. 12. One (patient "VA") was found to be an 8 bp (TTGTCCGT) insertion after nucleotide 714. This insertion created a shift in the reading frame and a truncated protein. The second patient ("CS") had an in-frame 3 bp deletions leading to the removal of amino acid 146 (isoleucine). Finally, patient "E" had an

in-frame 9 bp deletion (nucleotides 456 to 464) that resulted in the removal of three amino acids (Arg Val Val) at position 153-155. These combined results strongly support the conclusion that the g7 gene represents the VHL and the sporodic RCC tumor suppressor gene.

Example 7

Conservation of the g7 cDNA Across Species

In order to determine whether the g7 cDNA is highly conserved across species ranging from mammals to Drosophila and sea urchins, Zoo blotting using g7 cDNA as a probe was performed on DNA isolated from human (Homo sapiens), chimpanzee (Pan troglodytes), macaque (Macaca fascicularis), cow (Bovis domesticus), rat (Rattus norvigicus), mouse (Mus musculus), chicken (Gallus domesticus), frog (Xenopus laevis), fly (Drosophila melanogaster), sea urchin (Strongylocentrotus purpuratus), and yeast (Saccharomyces ceriviseae), all purchased from BIOS Laboratories (New Haven, CT, USA). (Pre)Hybridization was done in Church buffer [G. M. Church and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 1991 (1984)] at 65°C for 18 hours. Blots were washed in 0.1 x Church buffer at 60°C for 60 min. The results of the zoo blot are shown in Figure 6. The results demonstrate an extensive evolutionary conservation which is indicative of g7 serving a basic life function and also, of g7 having a tumor suppressor role.

Example 8

Identification and characterization of the promoter of the human VHL tumor supressor gene

Transcription initiation sites were located near the putative SPI/AP2 binding site. In one stably transfected clone of the renal carcinoma UMRC 6 cell line, the level of transcription from VHL minigene, containing 5′ flanking genomic DNA up to residue -647, was comparable with endogenous VHL expression. Using luciferase reporter

constructs which include 5' flanking genomic sequence (residues -467/+195) the minimal promoter was delineated within 106bp (positions -83/+23) in human embryonic kidney 293 cells. The 5' flanking DNA (residues -467/+195) were also examined for putative transcription factor binding sites and for other regulatory sequences. Several putative binding sequences for tissue specific transcription factors were located near transcription initiation sites. Among them is a core sequence for the Pax family of transcription factors which, apparently, regulates organogenesis. Pax 2 protein, a member of this family, is required for mesenchyme-to-epithelium conversion and is temporarily expressed during kidney development (Rothenpieler and Dressler, 1993). Since clear renal carcinomas originate from proximal tubular epithelium, Pax 2 may have an effect on VHL expression. A related gene, Pax 8, is also activated in developing kidney (Plachov, et al. 1990). Another potentially important site is a 12 bp consensus sequence for the nuclear respiratory factor 1 (NRF-1), which is involved in nuclear-mitochondrial interactions, and apparently, coordinates regulation of nuclear and mitochondrial genes during organelle biogensis (Evans and Scarpulla. 1990; Virbasius and Scarpulla 1994). Identical potential binding sites were also found in several other groups of genes (Virbasius, et al. 1993), including those involved in regulation of the cell cycle (*cdc 2, RCC 1*) cell growth (ornithine decarboxylase, DNA polymerase alpha) and apoptosis (bcl 2).

Consistently, all observed VHL point mutations were located downstream of the first (-68) methionine codon (Latif, et al., 1993b; Crossey, et al., 1994; Gnarra, et al. 1994; Richards, et al. 1994; Shuin, et al. 1994; Brauch, et al. 1995; Chen, et al. 1995) The codons upstream of this point are rarely used in human translated sequences (Wada, et al. 1992), whereas the downstream codons are used frequently. Finally, the region of homology between the

human VHL cDNA and its recently isolated mouse counterpart does not extend upstream of the first methionine (Latif and Duh. personal communication accession No. U12570).

To position the cloned cDNA within the full length VHL mRNA, RNase H mapping was employed (Berger, 1987). Restricted cleavage of the VHL mRNA with RNase H was directed by antisense DNA oligomers (Figures 7A-7C). The oligomers 1 and 2 were designed to anneal with the VHL mRNA at 267 to 296 nt and 572 to 596 nt downstream of the cDNA 5' end respectively (Figures 7A-7C). As shown on Figure 7A, the cleaved 5' part of the VHL mRNA is comparable by length with the known cDNA sequence. The size difference between 5' fragments obtained when RNA was digested with different oligomers agrees with the distance calculated from the cDNA sequence. Similar results were obtained using total RNA from 293, UMRC6, U2020 cell lines and human prostate poly(A) - RNA. Thus, the group 7 cDNA completely (or almost completely) represents the 5' end of the VHL mRNA.

In agreement with these data, extensive screening of 155 cDNA libraries (totalling 15 million clones. 100 positive clones were evaluated) and the rapid amplification of 5' cDNA end (5'RACE) technique did not yield any gain upstream of the known cloned cDNA sequences. No gross genomic rearrangements were found within the region covering 60 kb upstream of the VHL cDNA in more than 100 of the VHL kindred. When hybridized to Northern blots, the cloned genomic fragments from this region did not reveal any message the length of VHL.

Mapping of the Transcription Initiation Sites

Attempts to use primer extension to determine the VHL transcription starts were unsuccessful apparently because of high GC content and stable secondary structures near the 5' end of the VHL mRNA.

Thus, the transcription start sites were determined by RNase protection analysis. An antisense

riboprobe no. 1 (Figure 8A) was generated from PstI-NotI (530 nt) genomic fragment, which included a part of exon 1 from the cDNA sequence (223 nt) and the immediate 5' flanking region (308 nt). After hybridization with poly(A)$^-$ RNA from 293 cells several protected fragments 225 to 240 nt were found (Figure 8B slots 1, 2 and 3). This result roughly agrees with the RNase H mapping data but it falls far below the predicted figure (390 nt) for the "extended" exon 1 which would presumably contain the whole open reading frame, deducted from genomic sequence downstream of the putative splice acceptor site (Latif, et al., 1993b). To exclude any artifacts resulting from possible internal RNase cleavage of longer protected fragments, the experiment was repeated with probes no. 2 and no. 3. Probe no. 2, which was identical to probe no. 1 except for a shorter 5' flanking genomic region (44 nt instead of 308 nt) did not reveal any protected fragments (Figure 8B, slots 7, 8 and 9). The same results were obtained with poly(A)$^-$ RNA from human prostate and adult kidney (data not shown). According to these data transcription start sites were placed not more than 30 nt upstream of the 5' cloned cDNA 5' border.

For precise mapping of the transcription start sites, a shorter probe (no. 5; Figure 8A) was used which included 149 nt of the exon 1 sequences from the cDNA and 104 nt of the 5' flanking genomic region. Using RNA markers, the size of the protected fragments was identified as 152, 153, 161, 162, 163, 171 and 176 nts, which means that the 5' ends of the VHL mRNA were located respectively 3, 4, 12, 13, 14, 22 and 27 bp upstream of the cDNA border. The first nucleotide of the RNA specie which was initiated 22 bp upstream of the cDNA border was assigned number +1 (Figure 8C).

<u>A Functional Promoter is Located Around Initiation Sites</u>

To test the promoter activity a fragment from the 5' flanking genomic region (bases -467 through - 195) was

inserted into pGL-2-enhancer luciferase reporter vector, which was transfected into 293 cells. The fragment was shown to drive transcription of luciferase. The efficiency of the full length VHL promoter (bases -467-195) in 293 cells was assigned 100% SV 40 early promoter activity comprised 60% and thymidine kinase promoter - about 500 % of the full VHL promoter strength. The promoter activity appeared to be unidirectional, since the activity of the fragment in reverse orientation was about seven times weaker.

To localize more precisely the minimal promoter region, a set of 5′ and 3′ deletion constructs was prepared (Figure 9). The results of transfection indicated that the minimal promoter can be delineated within 106 bp, between restriction sites for EagI (-83) and SacII (-23). The minimal construct retained 32±9% of the full promoter activity. No separate promoter activity was found upstream of the EagI site (-83/-467). The region downstream of the SmaI site (+30/+195) enhances transcription by about two times; however it does not possess promoter activity of its own.

Because the mutations in the VHL gene apparently play a critical role in the origin of clear renal carcinoma (Latif, et al., 1993b; Gnarra et al. 1994; Shuin et al. 1994), the UMRC 6 cell line derived from this malignancy was also studied. When normalized to β-galactosidase expressed under cytomegalovirus (CMV) promoter, the luciferase activity in UMRC 6 cells was about two times lower than in 293 cells. However, the relative activity of different constructs compared to the full length construct no. 1 (Figure 3) in each cell line appeared to be similar. These data indicate that the same promoter region is active in both 293 and UMRC6 cell lines.

5' flanking genomic fragment, containing VHL promoter, confers apparently normal level of transcription to VHL minigenes

To estimate the level of transcription from the native VHL promoter in VHL minigenes in renal carcinoma, three minigene constructs were used, which were based on the pRc/CMV vector (Invitrogen). In these constructs CMV promoter/enhancer region was substituted by a VHL 5' flanking EcoRI-NotI genomic fragment which was fused to the rest of the VHL cDNA (Figure 10A). The final expression plasmids included VHL sequences from base -647 to +710 (pRcpVHL) and from -647 to +1664 (pRcpVHL3U). To eliminate any possible effects of the native VHL protein on cell growth, a frameshift was introduced into the VHL reading frame (duplication of bases -408/-412 in exon 2) of the pRcpVHL by digestion with BstEII, fill-in with Klenow fragment and relegation (plasmid pRcpVHLm). A transcript from the construct containing CMV promoter and VHL reading time (pRc-HAVHL) was used as a size marker of Northern blots. For transfection, the UMRC6 cell line was used. The cells were shown to have a 10 bp microdeletion in VHL exon 3 (Latif et al., 1993b) which would allow discrimination between endogenous and exogenous VHL mRNA by reverse transcription/polymerase chain reaction (RT-PCR). After transfection 40 to 50 geneticin positive clones were pooled and expression from VHL minigenes was assayed by Northern analyses (Figure 10B) and RT-PCR. The sizes of the exogenous VHL mRNAs indicated that transcription was initiated roughly from the same region inside the NotI-EcoRI fragment as we have shown above for endogenous VHL gene using the RNase protection assay. RT-PCR analysis confirmed expression from the VHL minigenes.

The question of whether the obvious difference in the level of expression between endo- and exogenes (Figure 10B) reflected a lack of important regulatory elements within the minigenes or just frequent rearrangements of the

VHL transgene in many of the geneticin resistant clones was next investigated. Five colonies were expanded and analysed by Southern and Northern blotting analyses (three of them were transfected by pRcpVHL3U construct, another two carried pRcpVHLm). However, only one clone (pRcpVHLm, clone 4) was shown to have nonrearranged VHL transgene (1.3 kb EcoRI fragment, Figure 11A) which expressed VHL mRNA (Figure 11B). Both the 950 nt and about 4800 nt transcripts showed a similar signal intensity on Northern blot with apparently the same gene copy number on Southern blot. This observation may indicate that the 5' VHL genomic region confers apparently normal level of transcription in the UMRC 6 renal carcinoma cell line. However, other factors may interfere, for example, the enhancing, (silencing) activity of the DNA sequences near integration site and different stability of the exogenous mRNA due to absence of a full-length 3' UTR.

<u>Sequence analysis of the VHL promoter</u>

The VHL promoter and exon 1 comprised a CpG island. The GC content within the minimal promoter region (-83/-23) is 72.6%. The minimal promoter harbors several GC-specific restriction sites including one for EagI, three for BssHII, one for SalI and six for HhaI. The region around minimal promoter (-467/-195) does not contain TATA and CCAAT boxes. A putative binding suite for SP-1 (KRGGCGKRRY; -1-13; Briggs, et al., 1986) and AP-2 transcription factors (YCSCCMNSS: -4/+13; Imagawa, et al. 1987) was found near transcription initiation sites. It appears to play a major role in the VHL transcription initiation. However, the reporter deletion analysis described above indicates that the region -83-10 is also functionally essential. Another site for SP1/AP2 was found in position +74/-83. Two sites for SP1 with a more loose recognition sequence (KRGGCKRRK; Faisst and Meyer, 1992) and one site for AP2 factor were located upstream of the minimal

promoter (Figure 6). Other putative transcription factor binding sites include Pax core sequence (GTTCC; -56/-60; Chaiepakis, et al., 1991) sites for nuclear respiratory factor 1 (YGCGCAYGCGCR: -92/-103; Evans and Scarpulla, 1990), nuclear hormone receptor for retinoic acid H-2RIIBP (GAGCTC; -21/-26; -293/-298; Marks, et al., 1992) and several other factors.

An important feature of the region further upstream to the VHL minimal promoter is a termination polyadenylation signal for RNA polymerase II (-384/- 379), which may prevent continuous transcription form other putative promoters upstream. Indeed, no evidence of such promoters has been found as yet.

The contents of all citations, i.e., journal articles, patents and the like, are incorporated herein by reference.

It is understood that the examples and embodiments described herein are for illustrative purposes and that various modifications and changes in light thereof to persons skilled in the art are included within the spirit and purview of this application and scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES

(ii) TITLE OF INVENTION: PARTIAL INTRON SEQUENCE OF VHL DISEASE GENE AND ITS USE IN DIAGNOSIS OF DISEASE

(iii) NUMBER OF SEQUENCES: 53

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: MORGAN & FINNEGAN, L.L.P.
(B) STREET: 345 PARK AVENUE
(C) CITY: NEW YORK
(D) STATE: NEW YORK
(E) COUNTRY: USA
(F) ZIP: 10154

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: FLOPPY DISK
(B) COMPUTER: IBM PC COMPATIBLE
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WORDPERFECT 5.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: MARCH 27, 1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/623,428
(B) FILING DATE: 28 MARCH 1996
(C) CLASSIFICATION: APPLICATION

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/061,889
(B) FILING DATE: 14 MAY 1993
(C) CLASSIFICATION: APPLICATION

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: FEILER, WILLIAM S.
(B) REGISTRATION NUMBER: 26,728
(C) REFERENCE/DOCKET NUMBER: 2026-4078PC2

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 758-4800
(B) TELEFAX: (212) 751-6849
(C) TELEX: 421792

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1816
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:

(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CCTCGCCTCC GTTACAACAG CCTACGGTGC TGGAGGATCC   40
TTCTGCGCAC GCGCACAGCC TCCGGCCGGC TATTTCCGCG   80
AGCGCGTTCC ATCCTCTACC GAGCGCGCGC GAAGACTACG  120
GAGGTCGACT CGGGAGCGCG CACGCAGCTC CGCCCCGCGT  160
CCGACCCGCG GATCCCGCGG CGTCCGGCCC GGGTGGTCTG  200
GATCGCGGAG GGAATGCCCC GGAGGGCGGA GAACTGGGAC  240
GAGGCCGAGG TAGGCGCGGA GGAGGCAGGC GTCGAAGAGT  280
ACGGCCCTGA AGAAGACGGC GGGGAGGAGT CGGGCGCCGA  320
GGAGTCCGGC CCGGAAGAGT CCGGCCCGGA GGAACTGGGC  360
GCCGAGGAGG AGATGGAGGC CGGGCGGCCG CGGCCCGTGC  400
TGCGCTCGGT GAACTCGCGC GAGCCCTCCC AGGTCATCTT  440
CTGCAATCGC AGTCCGCGCG TCGTGCTGCC CGTATGGCTC  480
AACTTCGACG GCGAGCCGCA GCCCTACCCA ACGCTGCCGC  520
CTGGCACGGG CCGCCGCATC CACAGCTACC GAGGTCACCT  560
TTGGCTCTTC AGAGATGCAG GGACACACGA TGGGCTTCTG  600
GTTAACCAAA CTGAATTATT TGTGCCATCT CTCAATGTTG  640
ACGGACAGCC TATTTTTGCC AATATCACAC TGCCAGTGTA  680
TACTCTGAAA GAGCGATGCC TCCAGGTTGT CCGGAGCCTA  720
GTCAAGCCTG AGAATTACAG GAGACTGGAC ATCGTCAGGT  760
CGCTCTACGA AGATCTGGAA GACCACCCAA ATGTGCAGAA  800
AGACCTGGAG CGGCTGACAC AGGAGCGCAT TGCACATCAA  840
CGGATGGGAG ATTGAAGATT TCTGTTGAAA CTTACACTGT  880
TTCATCTCAG CTTTTGATGG TACTGATGAG TCTTGATCTA  920
GATACAGGAC TGGTTCCTTC CTTAGTTTCA AAGTGTCTCA  960
TTCTCAGAGT AAAATAGGCA CCATTGCTTA AAAGAAAGTT 1000
AACTGACTTC ACTAGGCATT GTGATGTTTA GGGGCAAACA 1040
TCACAAAATG TAATTTAATG CCTGCCCATT AGAGAAGTAT 1080
TTATCAGGAG AAGGTGGTGG CATTTTTGCT TCCTAGTAAG 1120
TCAGGACAGC TTGTATGTAA GGAGGTTTAT ATAAGTAATT 1160
CAGTGGGAAT TGCAGCATAT CGTTTAATTT TAAGAAGGCA 1200
TTGGCATCTG CTTTTAATGG ATGTATAATA CATCCATTCT 1240
ACATCCGTAG CGGTTGGTGA CTTGTCTGCC TCCTGCTTTG 1280
GGAAGACTGA GGCATCCGTG AGGCAGGGAC AAGTCTTTCT 1320
CCTCTTTGAG ACCCCAGTGC CTGCACATCA TGAGCCTTCA 1360
GTCAGGGTTT CTCAGAGGAA CAAACCAGGG GACACTTTGT 1400
TAGAAAGTGC TTAGAGGTTC TGCCTCTATT TTTGTTGGGG 1440
GGTGGGAGAG GGGACCTTAA AATGTGTACA GTGAACAAAT 1480
GTCTTAAAGG GAATCATTTT TGTAGGAAGC ATTTTTTATA 1520
ATTTTCTAAG TCGTGCACTT TCTCGGTCCA CTCTTGTTGA 1560
AGTGCTGTTT TATTACTGTT TCTAAACTAG GATTGACATT 1600
CTACAGTTGT GATAATAGCA TTTTTGTAAC TTGCCATCCG 1640
CACAGAAAAT ACGAGAAAAT CTGCATGTTT GATTATAGTA 1680
TTAATGGACA AATAAGTTTT TGCTAAATGT GAGTATTTCT 1720
GTTCCTTTTT GTAAATATGT GACATTCCTG ATTGATTTGG 1760
GTTTTTTTGT TGTTGTTGTT TTGTTTTGTT TTGTTTTTTT 1800
GGGATGGAGG GAATTC                           1816
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 284
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: AMINO ACID SEQUENCE

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Pro Arg Leu Arg Tyr Asn Ser Leu Arg Cys Trp Arg
                 5                  10
Ile Leu Leu Arg Thr Arg Thr Ala Ser Gly Arg Leu
         15                  20
Phe Pro Arg Ala Arg Ser Ile Leu Tyr Arg Ala Arg
 25                  30                  35
```

```
Ala Lys Thr Thr Glu Val Asp Ser Gly Ala Arg Thr
            40                  45
Gln Leu Arg Pro Ala Ser Asp Pro Arg Ile Pro Arg
    50                  55                  60
Arg Pro Ala Arg Val Val Trp Ile Ala Glu Gly Met
                65                  70
Pro Arg Arg Ala Glu Asn Trp Asp Glu Ala Glu Val
        75                  80
Gly Ala Glu Glu Ala Gly Val Glu Glu Tyr Gly Pro
85                  90                  95
Glu Glu Asp Gly Gly Glu Glu Ser Gly Ala Glu Glu
            100                 105
Ser Gly Pro Glu Glu Ser Gly Pro Glu Glu Leu Gly
    110                 115                 120
Ala Glu Glu Glu Met Glu Ala Gly Arg Pro Arg Pro
                125                 130
Val Leu Arg Ser Val Asn Ser Arg Glu Pro Ser Gln
        135                 140
Val Ile Phe Cys Asn Arg Ser Pro Arg Val Val Leu
145                 150                 155
Pro Val Trp Leu Asn Phe Asp Gly Glu Pro Gln Pro
            160                 165
Tyr Pro Thr Leu Pro Pro Gly Thr Gly Arg Arg Ile
    170                 175                 180
His Ser Tyr Arg Gly His Leu Trp Leu Phe Arg Asp
                185                 190
Ala Gly Thr His Asp Gly Leu Leu Val Asn Gln Thr
        195                 200
Glu Leu Phe Val Pro Ser Leu Asn Val Asp Gly Gln
205                 210                 215
Pro Ile Phe Ala Asn Ile Thr Leu Pro Val Tyr Thr
            220                 225
Leu Lys Glu Arg Cys Leu Gln Val Val Arg Ser Leu
    230                 235                 240
Val Lys Pro Glu Asn Tyr Arg Arg Leu Asp Ile Val
                245                 250
Arg Ser Leu Tyr Glu Asp Leu Glu Asp His Pro Asn
        255                 260
Val Gln Lys Asp Leu Glu Arg Leu Thr Gln Glu Arg
265                 270                 275
Ile Ala His Gln Arg Met Gly Asp
            280
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 169
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
TACCCAACGC TGCCGCCTGG CACGGGCCGC CGCATCCACA    40
GCTACCGAGG TACGGGCCCG GCGCTTAGGC CCGACCCAGC    80
AGGACGATAG CACGGTCTAA GCCCCTCTAC CGCCCCGGGG   120
TCCATTCAGA CGGGGAACTA GGCCCCTTGA GGCAGGACAC   160
ATCCAGGGT                                     169
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 403
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
CTCCTGACCT CTATGATCCG CCTGCCTCGG CCTCCAAAGT     40
GCTGGGATTA CAGGTGTGGG CCACCGTGCC CAGCCACCGG     80
TGTGGCTCTT TAACAACCTT TGCTTGTCCC GATAGGTCAC    120
CTTTGGCTCT TCAGAGATGC AGGGACACAC GATGGGCTTC    160
TGGTTAACCA AACTGAATTA TTTGTGCCAT CTCTCAATGT    200
TGACGGACAG CCTATTTTTG CCAATATCAC ACTGCCAGGT    240
ACTGACGTTT TACTTTTTAA AAAGATAAGG TTGTTGTGGT    280
AAGTACAGGA TAGACCACTT GAAAAATTAA GCCCAGTTCT    320
CAATTTTTGC CTGATGTCAG GCACGGTATC CAATCTTTTT    360
GTATCCTATT CTCTACCATA AATAAAATGG AAGTGATGAT    400
TTT                                            403
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 193
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CTACAGAAGG CATGAACACC ATGAAGTGTC CATAGGGGCC     40
ACAGCATACA CACTGCCACA TACATGCACT CACTTTTTTT     80
CTTTAACCTA AAAGTGAAGA TCCATCAGTA GTACAGGTAG    120
TTGTTGGCAA AAGCCTCTTG TTCGTTCCTT GTACTGAGAC    160
CCTAGTCTGC CACTGAGGAT TTGGTTTTTG CCC           193
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 663

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
AGAGGCCAAG GCAGGAGGAT CACTTGAACC CAGGAGTTCG    40
AGACCAGCCT AGGCAACATA GCGAGACTCC GTTTCAAACA    80
ACAAATAAAA ATAATTAGTC GGGCATGGTG GTGCGCGCCT   120
ACAGTACCAA CTACTCGGGA GGCTGAGGCG AGACGATCGC   160
TTGAGCCAGG GAGGTCAAGG CTGCAGTGAG CCAAGCTCGC   200
GCCACTGCAC TCCAGCCCGG GCGACAGAGT GAGACCCTGT   240
CTCCAAAAAA AAAAAAAAAC ACCAAACCTT AGAGGGGTGA   280
AAAAAAATTT TATAGTGGAA ATACAGTAAC GAGTTGGCCT   320
AGCCTCGCCT CCGTTACAAC AGCCTACGGT GCTGGAGGAT   360
CCTTCTGCGC ACGCGCACAG CCTCCGGCCG GCTATTTCCG   400
CGAGCGCGTT CCATCCTCTA CCGAGCGCGC GCGAAGACTA   440
CGGAGGTCGA CTCGGGAGCG CGCACGCAGC TCCGCCCCGC   480
```

GTCCGACCCG CGGATCCCGC GGCGTCCGGC CCGGGTGGTC 520
TGGATCGCGG AGGGAATGCC CCGGAGGGCG GAGAACTGGG 560
ACGAGGCCGA GGTAGGCGCG GAGGAGGCAG GCGTCGAAGA 600
GTACGGCCCT GAAGAAGACG GCGGGGAGGA GTCGGGCGCC 640
GAGGAGTCCG GCCCGGAAGA GTC 663

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATAGTGGAAA TACAGTAACG AGTTGGCCTA GCCTCGC 37

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCCAGCTGGG TCGGGCCTAA GCGCCGGGCC CGT 33

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTGGCTCTTT AACAACCTTT GCTTGTCCCG ATA 33

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CAAGTGGTCT ATCCTGTACT TACCACAACA CCT 33

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TGTATACTCT GAAAGAGCGA TGCCTCCAGG T 31

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TACCATCAAA AGCTGAGATG AAACAGTGTA AGT 33

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:

(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGTGGAAATA CAGTAACGAG TTGGCCT 27

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:

(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAAATACAGT AACGAGTTGG CCTAGC 26

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTCCCAGTTC TCCGCCCTCC GGGGCAT 27

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TGGGTCGGGC CTAAGCGCCG GGCCCGT 27

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTTTAACAAC CTTTGCTTGT CCCGATA 27

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTGGCTCTTT AACAACCTTG C 21

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTCTATCCTG TACTTACCAC AACACCT 27

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CCTGTACTTA CCACAACACC TTAT 24

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CTGAGACCCT AGTCTGCCAC TGAGGAT 27

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TTCCTTGTAC TGAGACCCTA GT 22

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGAAATACAG TAACGAGTTG GCCT 24

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGAAATACAG TAACGAGTTG GCCTAGC 27

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:

(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ACGGGCCCGG CGCTTAGGCC CGACCCA 27

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:

(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ACGGGCCCGG CGCTTAGGCC CGACCCAGCA GG 32

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTGGCTCTTT AACAACCTTT GCTTGTCCCG ATA 33

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CTTTAACAAC CTTTGC 16

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GATAAGGTTG TTGTGGTAAG TACAGGA 27

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AGGTTGTTGT GGTAAGTACA GGATAGC 27

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CTCCTTGTAC TGAGACCCTA GT 22

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GTGAGACCCT AGTCTGCCAC TGAGGAT 27

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GAGGTCAC 8

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GATTGGTCAC 10

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: AMINO ACID SEQUENCE

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
Glu Glu Tyr Gly Pro Glu Glu Asp Gly Gly Glu Glu
                5                   10
Ser Gly
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: AMINO ACID SEQUENCE

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Gly Thr Gly Arg Arg Ile His Ser Tyr Arg Gly His
                 5                   10
Leu
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ACGACGCGCG GACTGCGATT GCAGAAGAT 29

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:

(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

AGCGACCTGA CGATGTCCAG TCTC 24

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:

(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CCTCGCCTCC GTTACAACA 19

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

```
GGATCCTAAT ACGACTCACT ATAGGGAGGC GCCCGACTCC    40
TCCC                                           44
```

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:

(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CTATCTAGAG GCCAAGGCAG GAGGATC 27

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:

(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CATTCTAGAT TCCCTCCGCG ATCCAGA 27

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CATTCTAGAC TCTTCCGGGC CGGACTC 27

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CACAAGTGAT GCCTTGTAGC TG 22

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CAGTAGTGTC CTGTATTTAG TG 22

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GTTGGCTATG GGTAGAATTG G 21

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CAGGGTAGCC TTGATCTAAG T 21

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GGAGGTCCTG AGAATATGTG TCC 23

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CAAAAGCTGA GATGAAACAG TGTAAGT 27

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GTTTGGTTAA CCAGAAGCCC ATCGT 25

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GATGGGCTTC TGGTTAACCA AACT 24

What is claimed is:

1. A purified and isolated VHL disease gene.

2. A purified and isolated VHL disease gene comprising a nucleic acid sequence according to SEQ. ID. NO: 1 and SEQ. ID. NO: 3 through SEQ. ID. NO: 6, and analogs thereof.

3. A purified and isolated VHL disease gene comprising a nucleic acid sequence according to SEQ. ID. NO: 1 and SEQ. ID. NO: 3 through SEQ. ID. NO: 6.

4. A purified and isolated VHL disease gene encoding a protein having an amino acid sequence according to SEQ. ID. NO: 2, or analogs thereof.

5. A method for detecting a mutant allele of a VHL gene in a subject suspected of having VHL disease or a disease related to the presence of the VHL gene mutation comprising:

analyzing DNA of a subject for the presence of said mutant allele.

6. The method of claim 5, wherein the VHL related disease is selected from the group consisting of sporadic renal cancer, uterine cancer, breast cancer, testicular cancer, bladder cancer, pancreatic cancer, ovarian cancer, lung cancer, adrenal tumors, brain tumors, lung tumors, or other cancers.

7. The method of claim 5, wherein said step of analyzing comprises Southern blot analysis.

8. The method of claim 7 wherein the probe used in said Southern blot analysis is derived from wild-type VHL disease gene sequence.

9. The method of claim 8, wherein said sequence is DNA.

10. The method of claim 9, wherein said DNA, or analogs thereof, has a sequence selected from the group consisting of SEQ. ID. NO: 1, and SEQ. ID. NO: 3 through SEQ. ID. NO: 6.

11. The method of claim 10, wherein said step of analyzing is carried out by PCR-SSCP.

12. The method of claim 11, wherein the primers used in PCR-SSCP are derived from DNA, or analogs thereof, having a DNA sequence selected from the group consisting of SEQ. ID. NO. 1, and SEQ. ID. NO: 3 through SEQ. ID. NO: 6.

13. The method of claim 12, wherein said primers have a nucleic acid sequence according to SEQ. ID. NO: 7 through SEQ. ID. NO: 34.

14. A method for detecting carriers of the VHL disease gene comprising:

analyzing DNA of a subject for mutations or alterations in VHL-specific DNA associated with VHL disease.

15. The method of claim 14, wherein said step of analyzing comprises PCR.

16. The method of claim 15, wherein primers used in said PCR are derived from a DNA sequence, or analogs thereof, having a DNA sequence selected from the group consisting of SEQ. ID. NO: 1, and SEQ. ID. NO: 3 through SEQ. ID. NO: 6.

17. The method of claim 16, wherein said primers have a sequence according to SEQ. ID. NO: 7 through SEQ. ID. NO:

34.

18. Primers derived from the VHL disease gene sequence.

19. The primers of claim 18, wherein said primers, or analogs thereof, have a DNA sequence according to SEQ. ID. NO. 1 and SEQ. ID. NO: 3 through SEQ. ID. NO. 6.

20. The primers of claim 19, wherein said primers have nucleic acid sequences selected from the group consisting of SEQ. ID. NO: 7 through SEQ. ID. NO: 34.

21. A diagnostic kit for use in detecting carriers of the VHL disease gene, said fit comprising:

primers having nucleic acid sequences selected from the group consisting of SEQ. ID. NO: 7 through SEQ. ID. NO: 34.

22. A method for detecting carriers of the VHL disease gene comprising:

analyzing the protein of a subject for alterations in VHL protein expression associated with VHL disease.

23. The method of claim 22, wherein said step of analyzing comprises Western blotting.

24. The method of claim 23, wherein the antibody used in said Western blotting is directed against VHL protein.

25. A recombinant VHL protein derived from the gene sequence of claim 2.

26. An antibody to the VHL protein or an immunogenic fragment thereof.

27. The antibody of claim 26, wherein said antibody is selected from the group consisting of polyclonal and monoclonal antibodies.

FIG. 1

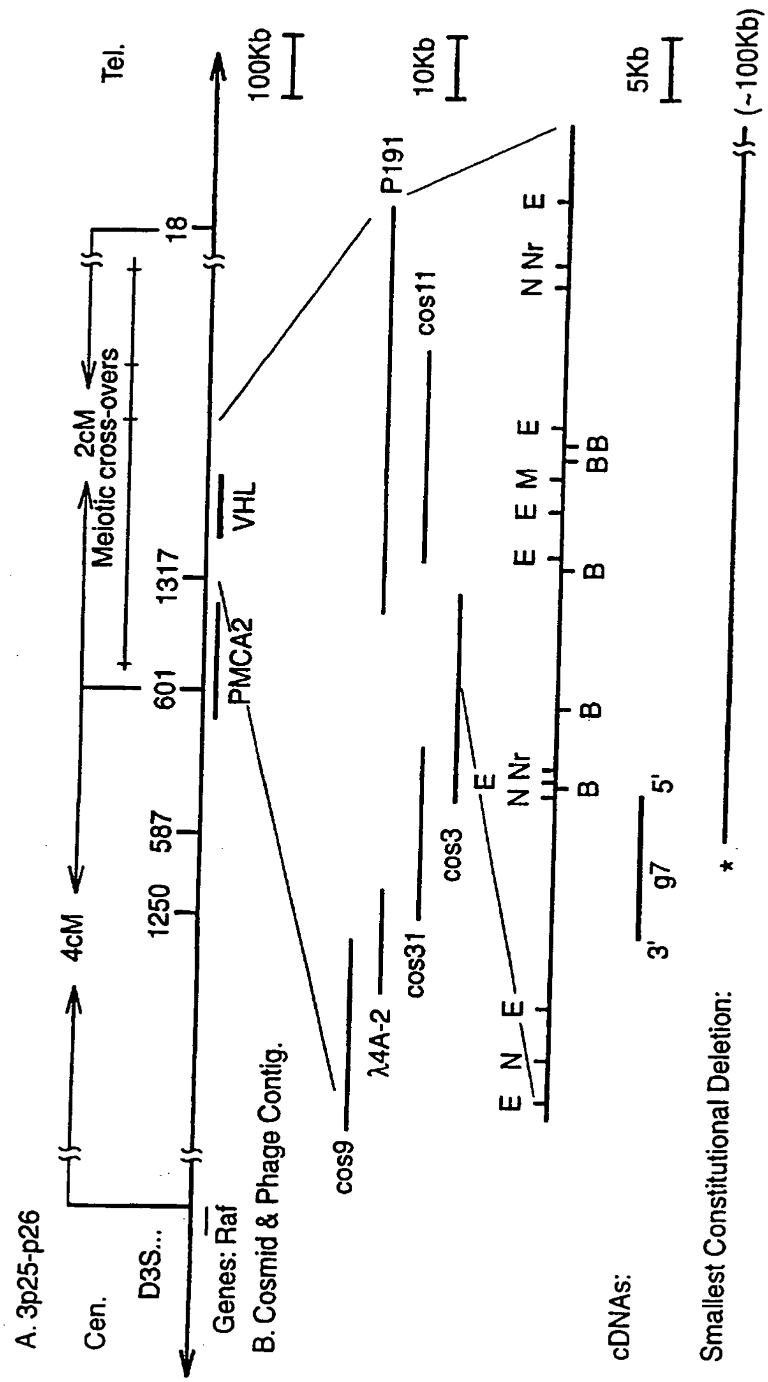

A. 3p25-p26
Cen.
Tel.
D3S...
1250
587
601
1317
18
4cM
2cM
Meiotic cross-overs
100Kb
Genes: Raf
PMCA2
VHL
B. Cosmid & Phage Contig.
cos9
λ4A-2
cos31
cos3
cos11
P191
10Kb
5Kb
cDNAs:
3'
g7
5'
Smallest Constitutional Deletion:
(~100Kb)

FIG. 2A

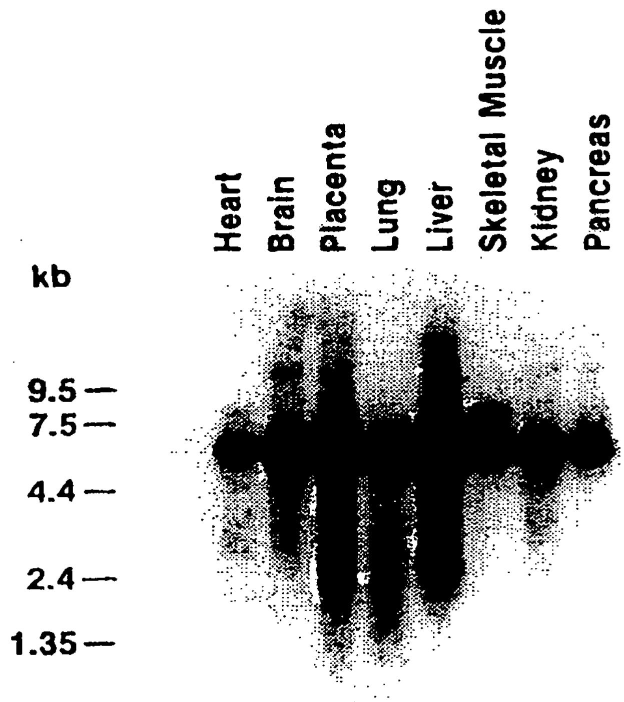

Heart
Brain
Placenta
Lung
Liver
Skeletal Muscle
Kidney
Pancreas
kb
9.5
7.5
4.4
2.4
1.35

FIG. 2B

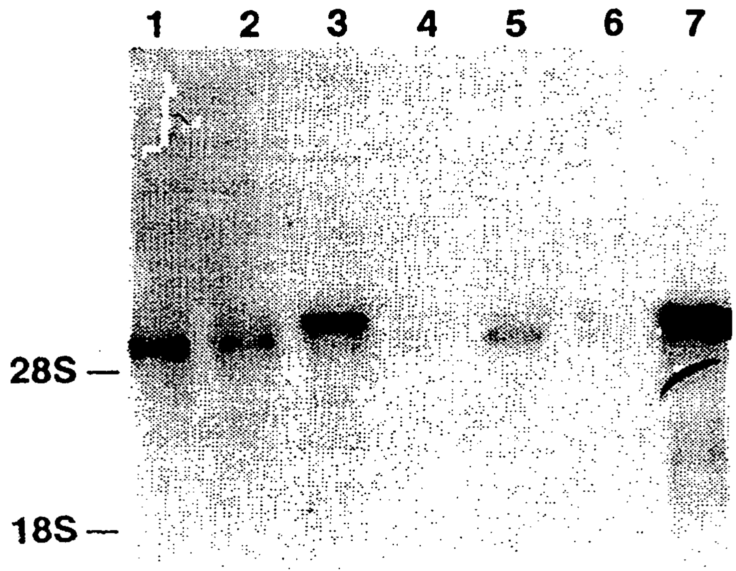


FIG. 3A

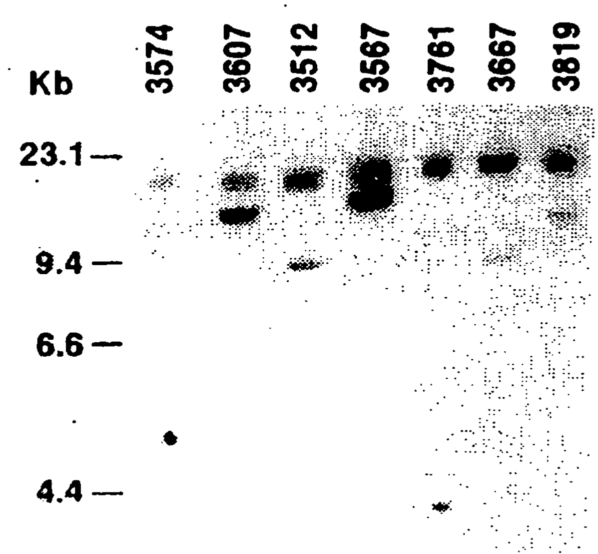
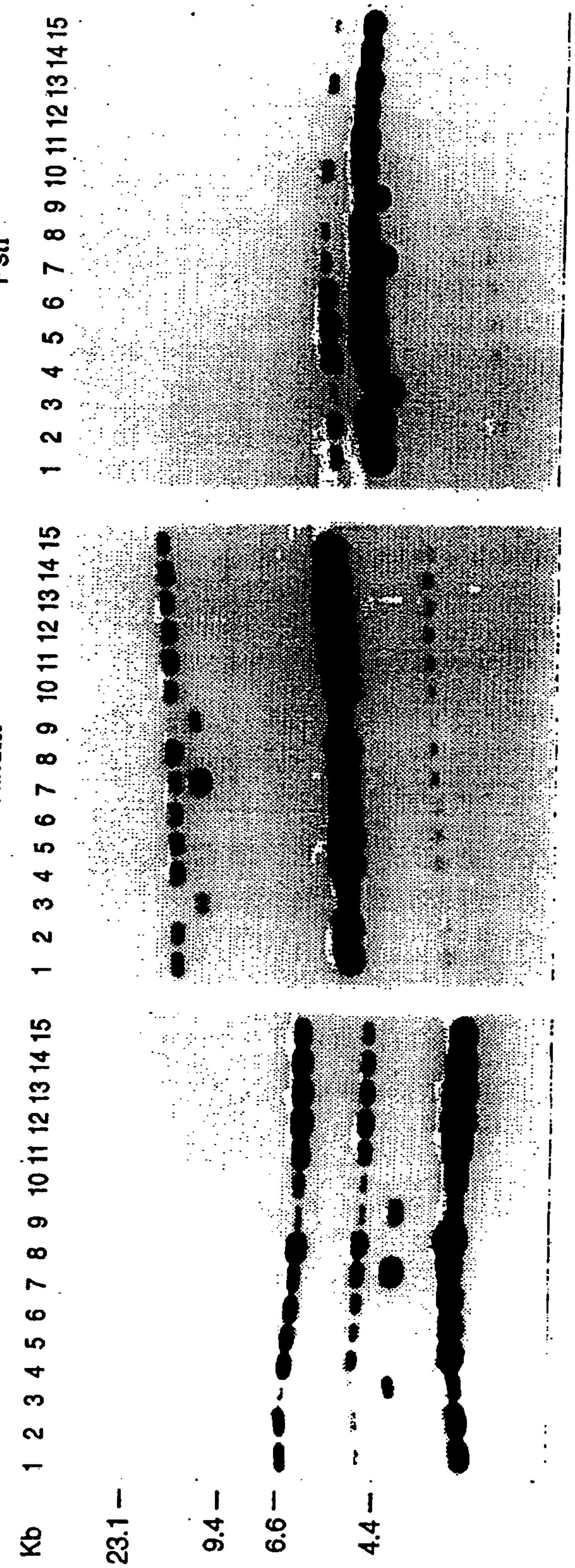


FIG. 3B


DraI
HindIII
PstI
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Kb
23.1
9.4
6.6
4.4

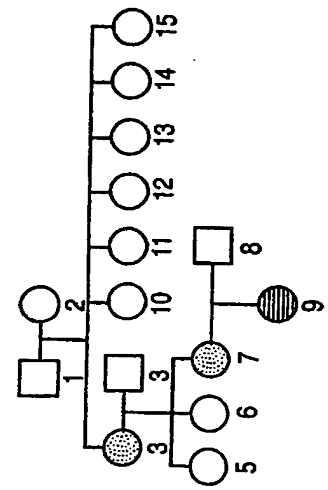

1
2
3
3
5
6
7
8
9
10
11
12
13
14
15

FIG. 3C

FIG. 3D

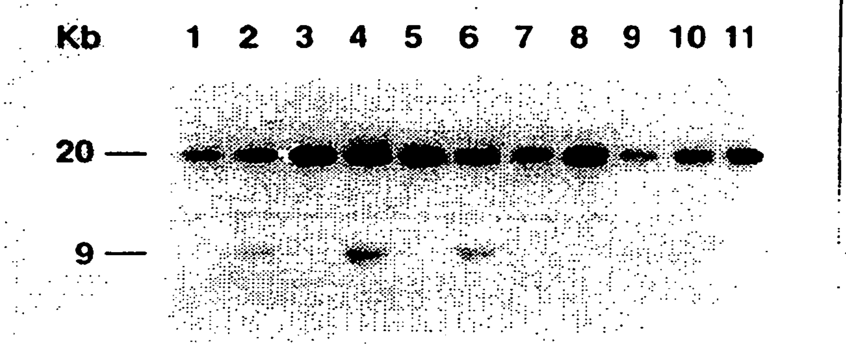


FIG. 3E

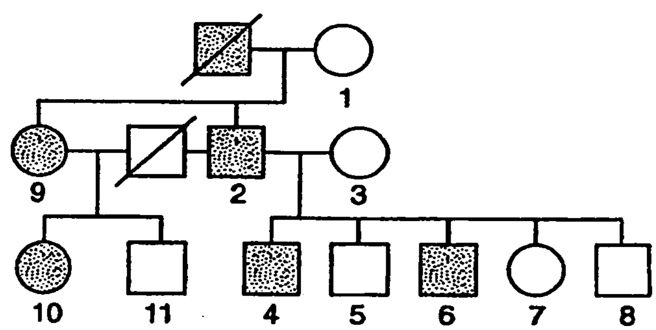


FIG. 4

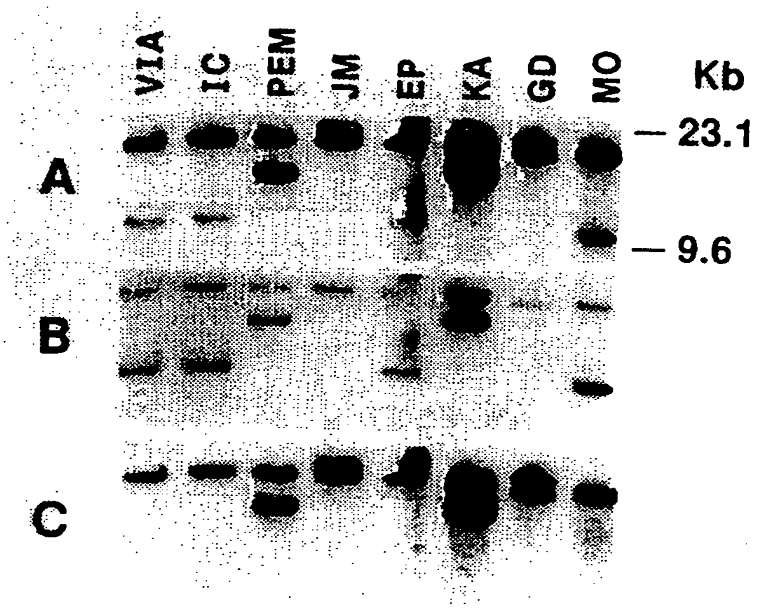


FIG. 5B

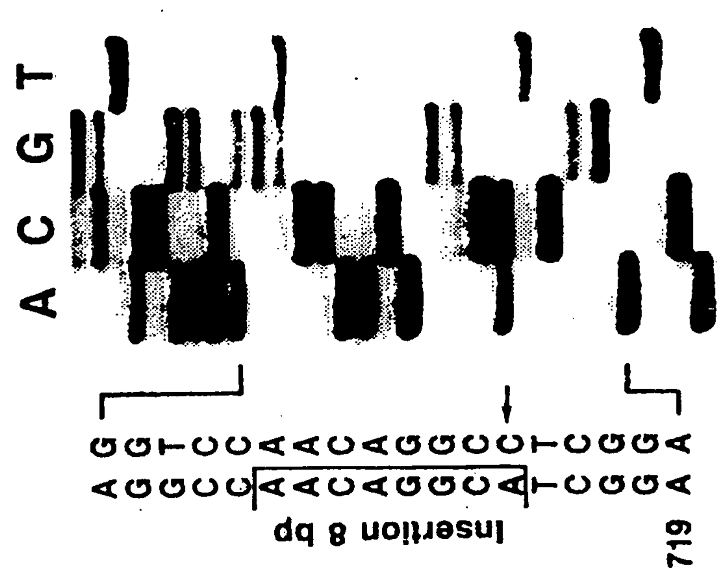
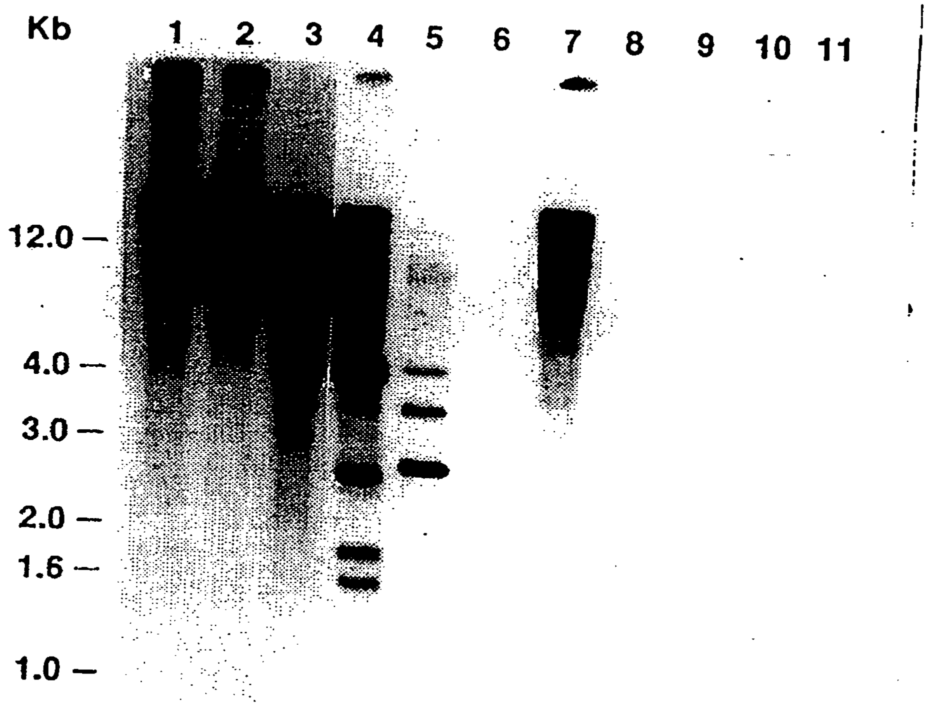

A C G T
GGTCCAACAGGCCTCGGA
AGGCCAACAGGCATCGGA
Insertion 8 bp
719

FIG. 5A


A C G T
702
GGTCCAACAGGCCTCGGA
714
719

FIG. 6



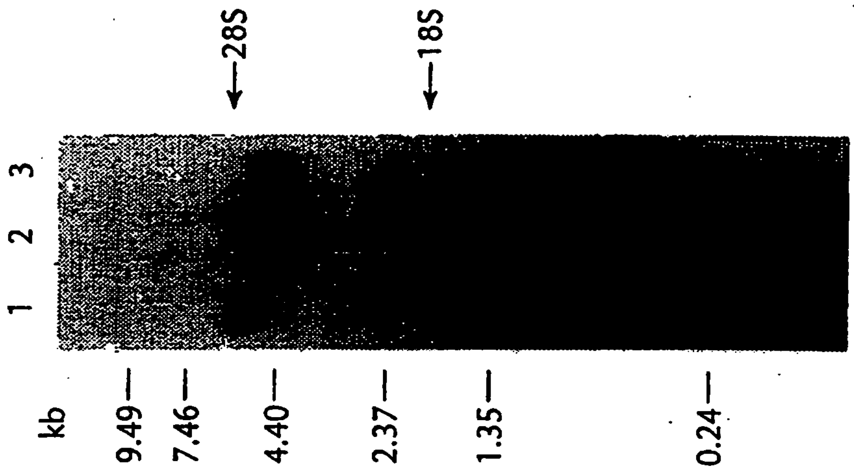

28S
18S
1
2
3
kb
9.49
7.46
4.40
2.37
1.35
0.24

FIG. 7B

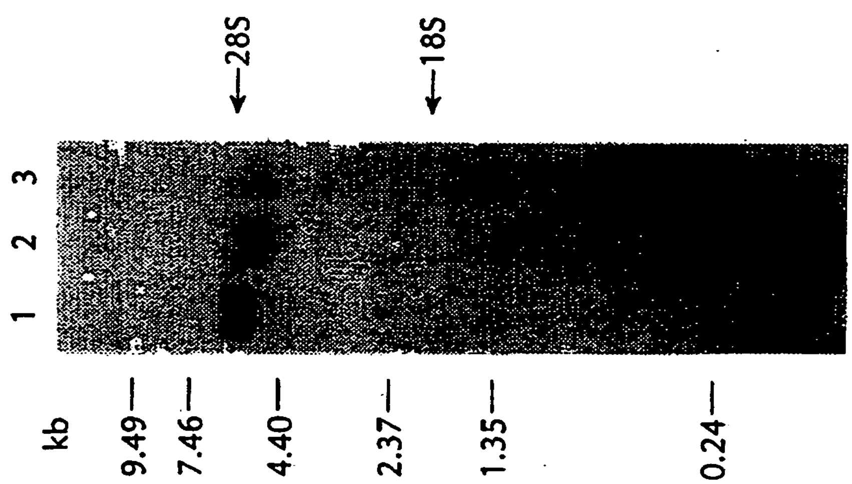

28S
18S
1
2
3
kb
9.49
7.46
4.40
2.37
1.35
0.24

FIG. 7A

FIG. 7C

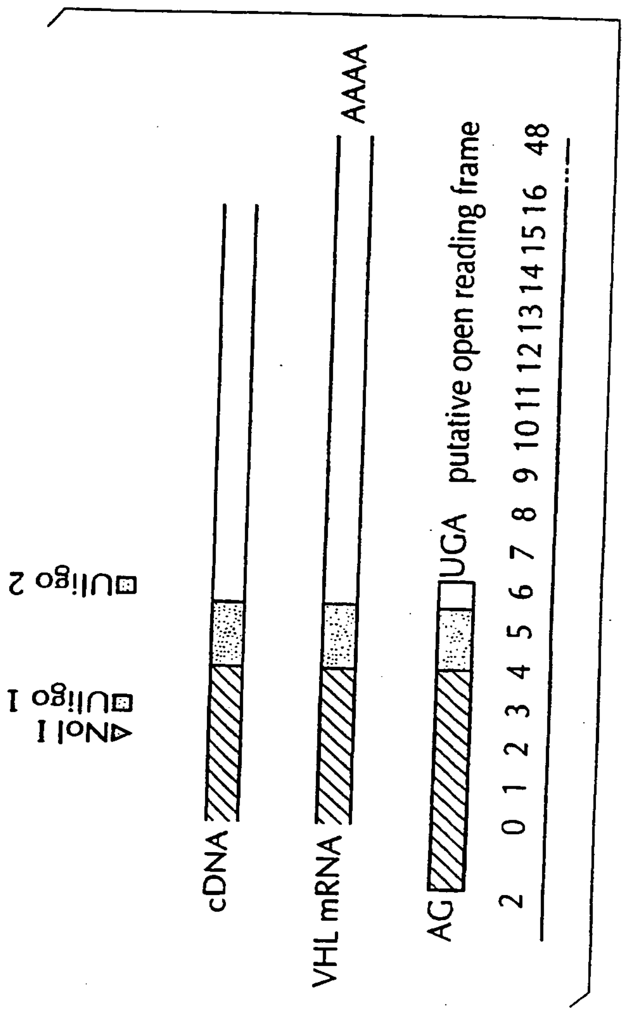

Oligo 1
Oligo 2
cDNA
VHL mRNA
AAAA
AG
UGA
putative open reading frame
2 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 48

FIG. 8A

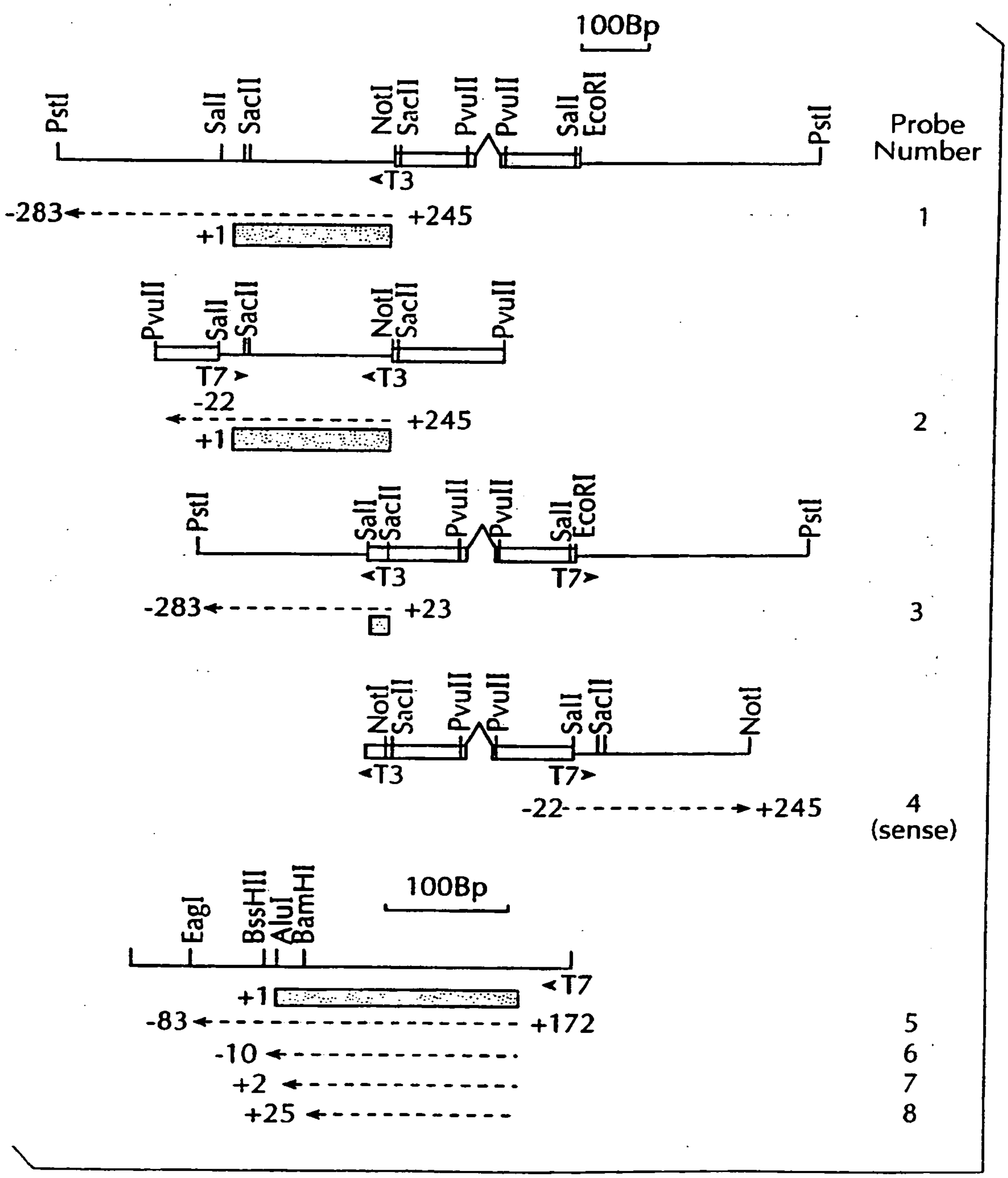

100Bp
PstI
SalI
SacII
NotI
SacII
PvuII
PvuII
SalI
EcoRI
PstI
Probe Number
T3
-283
+245
+1
1
PvuII
SalI
SacII
NotI
SacII
PvuII
T7
T3
-22
+245
+1
2
PstI
SalI
SacII
PvuII
PvuII
SalI
EcoRI
PstI
T3
T7
-283
+23
3
NotI
SacII
PvuII
PvuII
SalI
SacII
NotI
T3
T7
-22
+245
4 (sense)
EagI
BssHII
AluI
BamHI
100Bp
T7
+1
-83
+172
5
-10
6
+2
7
+25
8

FIG. 8B

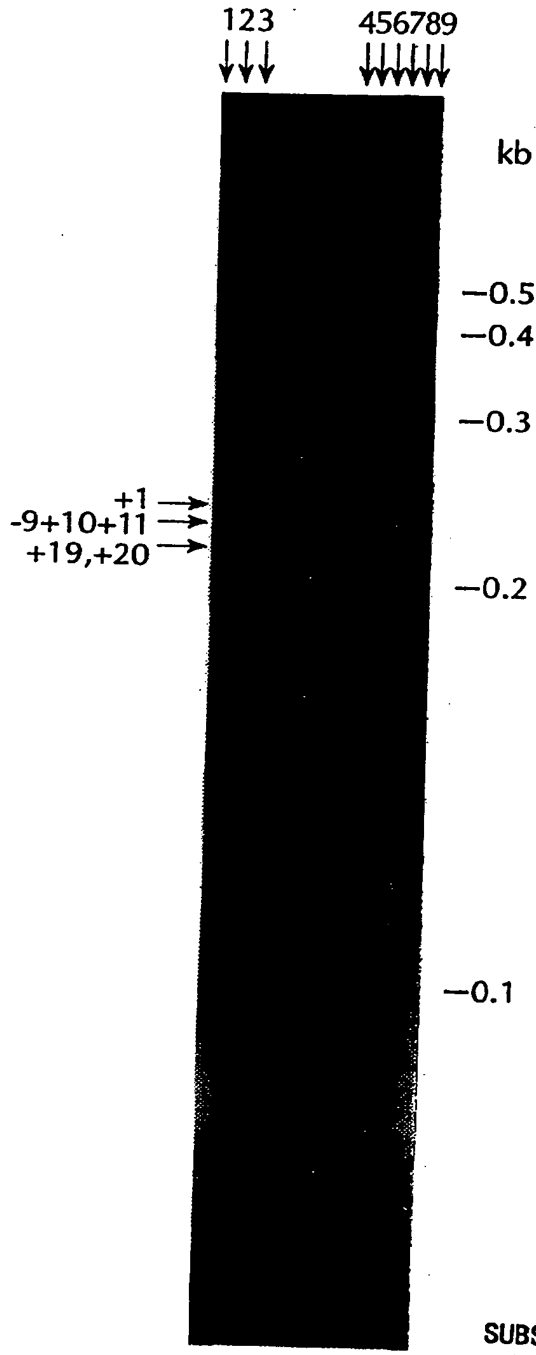

123
456789
kb
—0.5
—0.4
—0.3
+1
-9+10+11
+19,+20
—0.2
—0.1

FIG. 8C

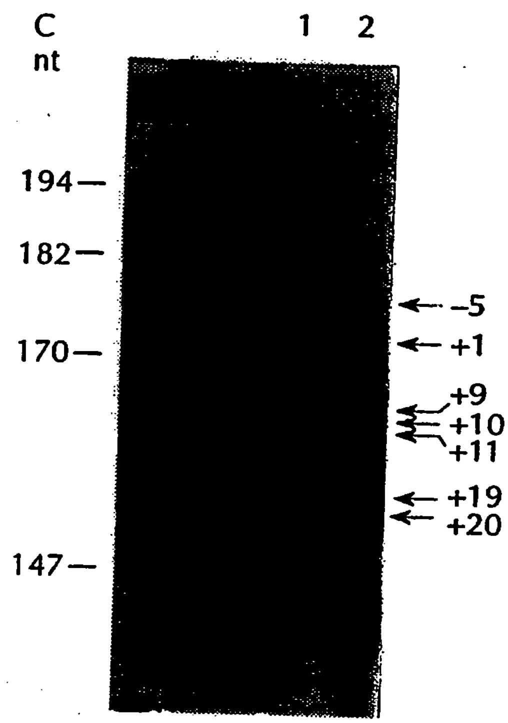
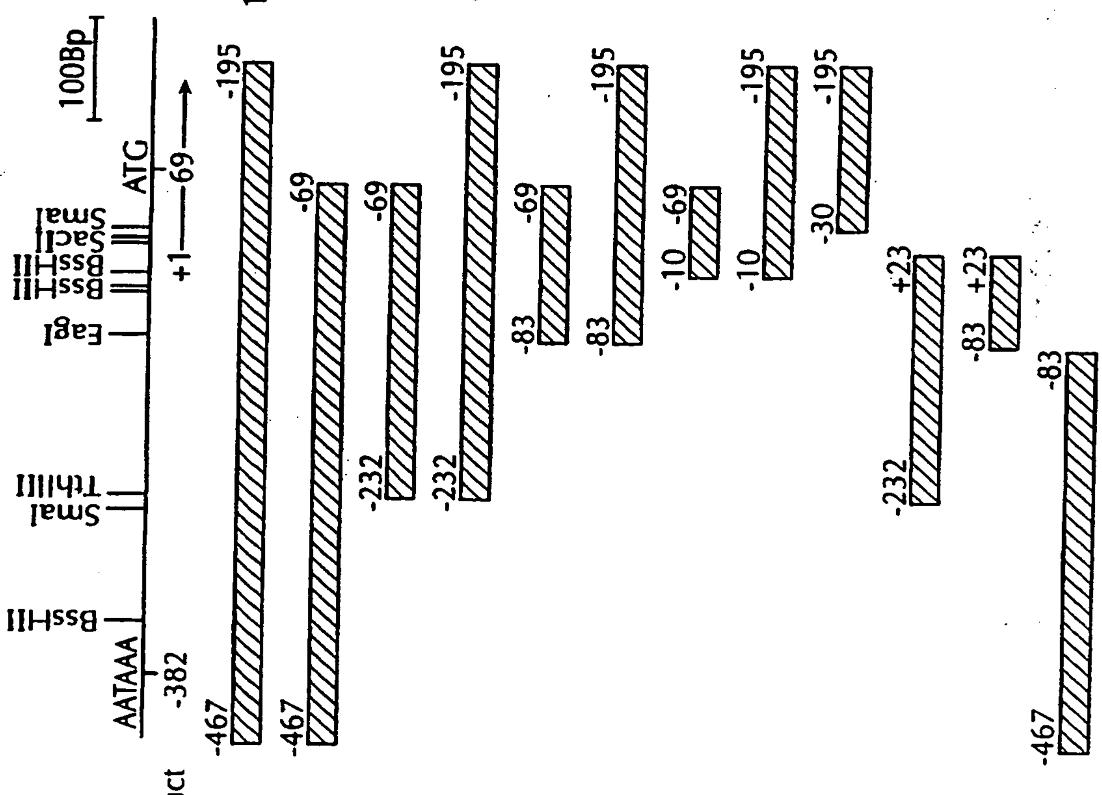

C
nt
1
2
194—
182—
170—
147—
-5
+1
+9
+10
+11
+19
+20

FIG. 9



Construct No.	Luciferase Activity±SE 293	Luciferase Activity±SE UMRC6
1	100±18%	45±8%
2	44±10%	ND
3	48±12%	22±5%
4	90±15%	41±6%
5	36±5%	N/D
6	51±9%	35±8%
7	2±3%	N/D
8	8±3%	2±2%
9	5±4%	N/D
10	36±8%	15±3%
11	32±9%	16±5%
12	6±3%	N/D

FIG. 10A

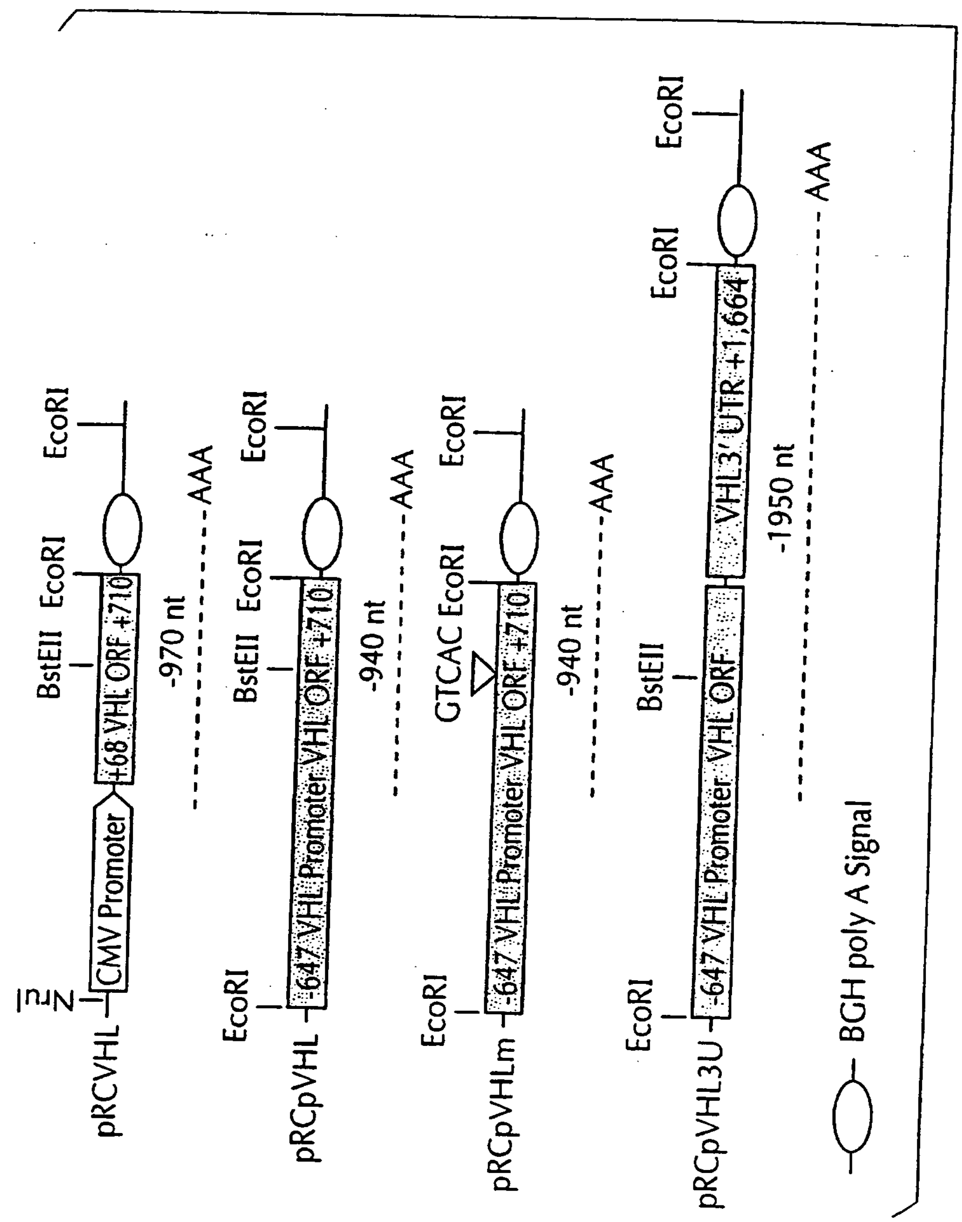

pRCVHL
NruI
CMV Promoter
+68 VHL ORF +710
BstEII
EcoRI
EcoRI
-970 nt
AAA
pRCpVHL
EcoRI
-647 VHL Promoter VHL ORF +710
BstEII
EcoRI
EcoRI
-940 nt
AAA
pRCpVHLm
EcoRI
-647 VHL Promoter VHL ORF +710
GTCAC
EcoRI
EcoRI
-940 nt
AAA
pRCpVHL3U
EcoRI
-647 VHL Promoter VHL ORF
BstEII
VHL3' UTR +1,664
EcoRI
EcoRI
-1950 nt
AAA
BGH poly A Signal

FIG. 10B

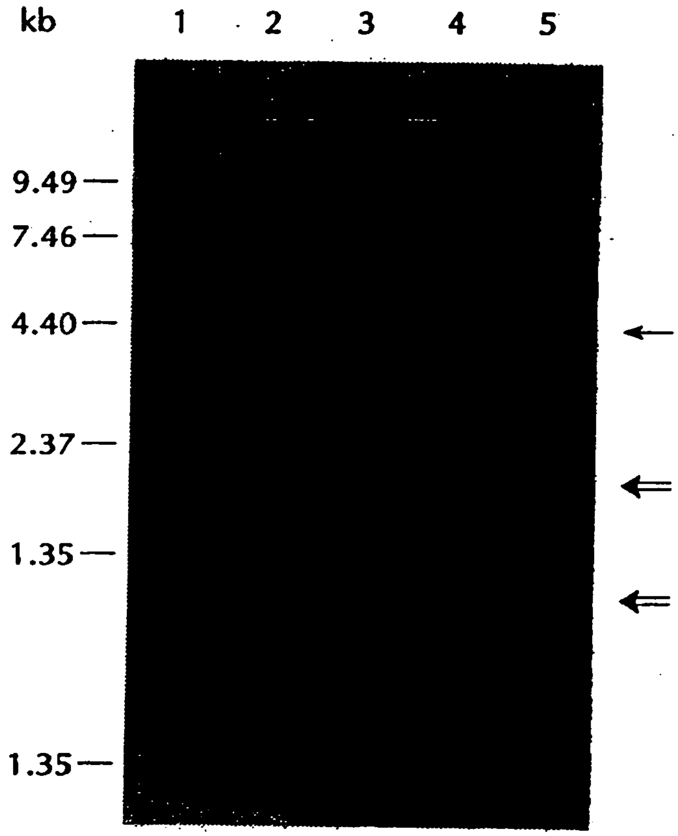


FIG. 11B

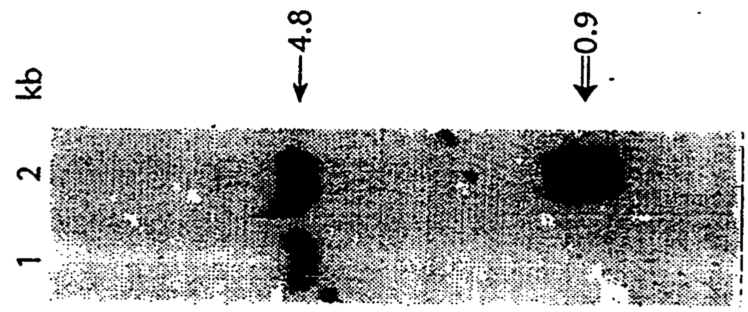


FIG. 11A

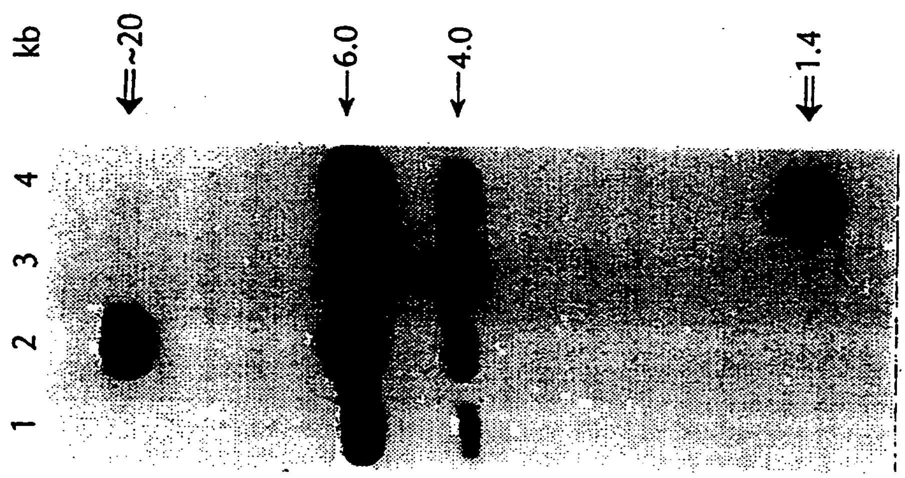


FIG. 12

```
-468  AGAGGCCAAG GCAGGAGGAT CACTTGAACC CAGGAGTTCG
          SP1
-428  AGACCAGCCT AGGCAACATA GCGAGACTCC GTTTCAAACA
         term                                BssHII
-388  ACAAATAAAA ATAATTAGTC GGGCATGGTG GTGCGCGCCT
                                 SP1
-348  ACAGTACCAA CTACTCGGGA GGCTGAGGCG AGACGATCGC

-308  TTGAGCCAGG GAGGTCAAGG CTGCAGTGAG CCAAGCTCGC
                        SmaI AP-2          Tth 111 I
-268  GCCACTGCAC TCCAGCCCGG GCGACAGAGT GAGACCCTGT

-228  CTCCAAAAAA AAAAAAAAAC ACCAAACCTT AGAGGGGTGA

-188  AAAAAAATTT TATAGTGGAA ATACAGTAAC GAGTTGGCCT

-148  AGCCTCGCCT CCGTTACAAC AGCCTACGGT GCTGGAGGAT
                                    EagI
-108  CCTTCTGCGC ACGCGCACAG CCTCCGGCCG GCTATTTCCG
                                  BssHII BssHII
 -68  CGAGCGCGTT CCATCCTCTA CCGAGCGCGC GCGAAGACTA
          SalI              BssHII     +1 SP1/AP2
 -28  CGGAGGTCGA CTCGGGAGCG CGCACGCAGC TCCGCCCCGC
          SacII        SacII            SmaI
 +13  GTCCGACCCG CGGATCCCGC GGCGTCCGGC CCGGGTGGTC
                                  SP1/AP2
 +53  TGGATCGCGG AGGGAATGCC CCGGAGGGCG GAGAACTGGG

 +93  ACGAGGCCGA GGTAGGCGCG GAGGAGGCAG GCGTCGAAGA
                                             NarI
+133  GTACGGCCCT GAAGAAGACG GCGGGGAGGA GTCGGGCGCC

+173  GAGGAGTCCG GCCCGGAAGA GTC
```

FIG. 13

Exon 1
5'
TACCCAACGCTGCCGCCTGGCACGGGCCGCCGCATCCACAGCTACCGAGgtac
gggcccggcgcttaggcccgacccagcaggacgatagcacggtctaagcccctctaccgccccggggtccattcagacggg
gaactaggcccccttgaggcaggacacatccagggt

Exon 2
5'
ctcctgacctctatgatccgcctgcctcggcctccaaagtgctgggattacaggtgtgggccaccgtgcccagccaccggtGT
GGCTCtttaacaacctttgcttgtcccgatagGTCACCTTTGGCTCTTCAGAGATGCAGGGAC
ACACGATGGGCTTCTGGTTAACCAAACTGAATTATTTGTGCCATCTCTCAAT
GTTGACGGACAGCCTATTTTTGCCAATATCACACTGCCAGgtactgacgttttactttttaa
aaagataaggttgttgtggtaagtacaggatagaccacttgaaaaattaagcccagttctcaatttttgcctgatgtcaggcacggt
atccaatctttttgtatcctattctctaccataaataaaatggaagtgatgatttt

Exon 3
5'
ctacagaaggcatgaacaccatgaagtgtccataggggccacagcatacacactgccacatacatgcactcactttttttctttaa
cctaaaagtgaagatccatcagtagtacaggtagttgttggcaaaagcctcttgttcgttccttgtactgagaccctagtctgccact
gaggatttggtttttgccc

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/05059

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12Q1/68 C07K14/47 C07K16/18 G01N33/574
G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 26894 A (US HEALTH) 24 November 1994	1,5-9, 14,15, 18,22-27
A	see the whole document	2-4, 10-13, 16,17, 19-21

X	JAPANESE JOURNAL OF CANCER RES, vol. 86, no. 10, October 1995, pages 905-9, XP000677118 KIKUCHI Y ET AL: "Cloning of the rat homologue of the Von Hippel-Lindau tumour suppressor gene and its non-somatic mutation in rat renal cell carcinomas " see the whole document	1

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
27 June 1997	14.07.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Osborne, H

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In. ...onal Application No
PCT/US 97/05059

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HUMAN MUTATION, vol. 5, no. 1, 1995, pages 66-75, XP000677115 CHEN F ET AL: "Germline mutations in Von Hippel-Lindau disease tumor suppressor gene: Correlations with phenotype" whole document, especially page 68, lns 7 & 8. ---	5-21
A	INTERNATIONAL JOURNAL OF CANCER, vol. 60, May 1995, pages 437-38, XP000677121 SUN Y ET AL: "The Von Hippel-Lindau (VHL) disease tumor -suppressor gene is not mutated in Nasopharyngeal carcinomas" see the whole document -----	5-21

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/05059

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9426894 A	24-11-94	AU 6833794 A	12-12-94
		EP 0698093 A	28-02-96
		JP 8511158 T	26-11-96